GM604 – A Multiple-Target Regulator that Provides a Novel Therapeutic Strategy for Treatment of ALS and Other Neurodegenerative Diseases

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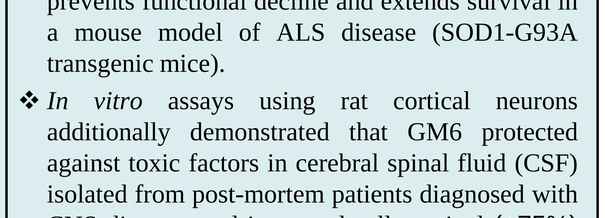
ABSTRACT



- Amyotrophic lateral sclerosis (ALS) is a fatal heterogeneous neurodegenerative disease that lacks effective treatment options. Genervon hypothesized that the traditional single-target approach to treat neurodegenerative disease may not be effective due to the complexity of these conditions and their multifactorial pathogenesis.
- Genervon Biopharmaceuticals has discovered and developed GM604 (GM6), a 6-amino acid peptide drug modeled upon an endogenous embryonic stage neurotrophic factor isolated from the developing nervous system.
- Using a systems biology approach, we discovered that GM6 has multi-target effects consistent with a neurotrophic factor, leading to improved survival of neurons in neurodegenerative patients.
- Through *in vivo* studies, we have shown that GM6 prevents functional decline and extends survival in a mouse model of ALS disease (SOD1-G93A transgenic mice).
- *In vitro* assays using rat cortical neurons additionally demonstrated that GM6 protected against toxic factors in cerebral spinal fluid (CSF) isolated from post-mortem patients diagnosed with CNS diseases and increased cell survival (+75%) from CSF derived from ALS patients significantly.
- Our pilot phase 2A clinical trial of only two weeks (6 doses) is safe and suggested a positive signal of GM6 in ALS patients with favorable shifts in biomarkers of neurodegenerative diseases. GM6 decreased plasma levels of ALS biomarkers (TDP-43, P = 0.008; Tau, P = 0.037; SOD1, P = 0.009). We also observed site-specific improvement in forced vital capacity (P=0.027).
- RNA-seq was used to determine whether ALS-associated genes exhibit unique responses to GM6 treatment in SH-SY5Y cells, which would suggest that GM6 is impacting signaling pathways linked to processes that underlie ALS onset and/or progression. Bioinformatic analyses identified GM6-regulated genes with potentially important roles in ALS development and progression. These genes were categorized with respect to 6 hypothesized mechanisms of action.

INTRODUCTION

- Multiple pathologies are common in neurodegenerative disease, and there is considerable overlap in pathways and targets driving neurodegenerative diseases.
- Taking a systems biology approach, Genervon discovered an endogenous embryonic stage regulator Motoneuronotrophic Factor (MNTF). (Yu et al. 2008, Brain Res 1238: 143-53)
- MNTF has been shown to activate the Insulin Receptor, mediate the differentiation of murine embryonic stem cells, promote axonogenesis and motor neuron regeneration (rat sciatic and femoral nerves).
- Genervon has advanced to the clinic GM6 –a 6 amino acid active analogue peptide of MNTF.

OBJECTIVE

- To test the hypothesis that GM6 triggers developmental-stage cascades to ultimately encourage neuron survival and attenuate progression of neurodegenerative diseases.
- Use a systems biology approach to evaluate whether GM6 has multi-target effects consistent with a neurotrophic factor, leading to improved survival of neurons in neurodegenerative patients.
- Evaluate whether GM6 protects against toxic factors in cerebral spinal fluid (CSF) isolated from patients diagnosed with CNS diseases and increases cell survival significantly.
- Identify ALS-associated genes for which expression is altered by GM6 in SH-5YSY neuroblastoma cells (RNA-seq).
- Design clinical trials to evaluate the clinical significance of these findings and ultimately develop a tentative mechanism of action for use of GM6 as a treatment for ALS and other neurodegenerative diseases.

METHODS

Neuroprotection of rat cortical neurons from cytotoxic molecules present at high levels in the postmortem human cerebrospinal fluid samples from ALS patients

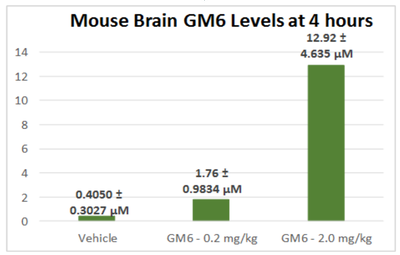
The UCLA Brain Center supplied human patient cerebrospinal fluid (CSF) samples from patients with 8 different neurological disorders (including ALS patients) and healthy control subjects. We tested the effects of CSF samples on neuronal cell death, and evaluated whether GM6 prevented death or cellular injury.

Detecting GM6 in brain

C57BL6 mice were injected with a single bolus IV tail vein injection of GM6 at 0.2 and 2.0 mg/kg

At four hours, the animals were sacrificed, and half of the brain was frozen for ELISA analysis

ELISA assay with the supernatant from the brain homogenate detected GM6 at statistically significant levels, at all doses, compared to control (P = 0.0001)



GM6 rapidly transits the Blood Brain Barrier

GM6 in ALS mouse model (SOD1 transgenic mice)

SOD-G93A transgenic mice were injected with vehicle (control) or GM604 intravenously at day 80 and daily thereafter until sacrifice. Clinical scores for each animal were tallied every third day after treatment. Onset of disease is determined by a clinical score of 1 or higher

Probability of onset: Probability of disease onset is defined as the number of mice with disease onset divided by the total number of animals in each study group.

Probability of death: Probability of death is defined as the number of mice that died divided by the total number of animals in each study group.

Improvement of functions in the SOD1 model of ALS

Rotarod test: The ability to remain on the rotarod was tested every third day after treatment. The average value of the time each animal remained on the rotating rod for each study group is plotted as a function of age.

Grip Strength: Grip strength was measured every third day after treatment. The average value of grip strength for each study group is plotted as a function of age.

Clinical scores: Clinical scores were tallied every third day after treatment: No sign of weakness (0); tremor and loss of splay reflex (1); paresis in one hindlimb (2); paresis in both hindlimbs (3); paralysis of one or both hindlimbs (4). The average score for each study group is plotted as a function of age.

Phase 2A Clinical Study

With the efficacy results in the SOD1 animal model, Genervon conducted a 2-centers Phase 2A, double-blind, randomized, placebo-controlled pilot trial with GM604 in 12 patients with Familial or Sporadic ALS diagnosed as definite ALS according to the El Escorial Criteria (ALS Protocol GALS-001). Definite ALS patients with ALS disease onset within 24 months and FVC >65% were included. Patients were randomized at each site to four GM604 treated and two placebos treated. Patients received 6 doses of GM604 or placebo, administered as slow IV bolus injections (320 mg) three times per week for 2 consecutive weeks. Objectives were to assess the safety and efficacy of GM604 based on ALSFRS-R, FVC and selected biomarkers (TDP-43, Tau and SOD1, pNFH).

Bioinformatic analysis and mechanism of action

Gene expression analyses are performed using RNA-seq. Two sets of experiments were performed independently by laboratories at Sunny Biodiscovery (Santa Paula, CA) and SBH Sciences (Natick, MA) (Table 1). In each experiment, SH-5YSY cells were treated with GM604 for varying lengths of time (6-48 hours; *n*=3-5 replicates per treatment). Goals were to determine whether ALS-associated genes exhibit unique responses to GM6 treatment, which would suggest that GM6 is impacting on signaling pathways linked to processes that underlie ALS onset and/or progression.

Table 1. Expression profiling datasets. In all experiments, GM6 was applied to cells at a concentration of 1 mg/ml in type 1 water.

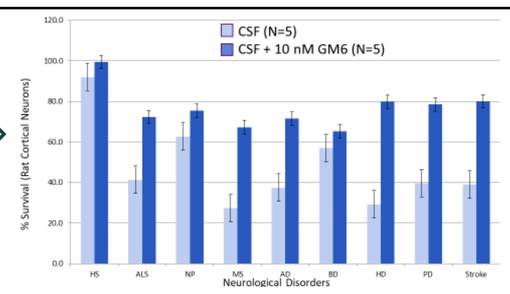
Experiment No.	Laboratory	GM6 Treatment Time
1[a]	Sunny Biodiscovery	6 hours
2[a]	Sunny Biodiscovery	24 hours
3[a]	Sunny Biodiscovery	48 hours
4[b]	SBH Sciences	6 hours
5[b]	SBH Sciences	24 hours

[a] Sequencing performed by University of Michigan core facility (Ann Arbor, MI).
[b] Sequencing performed by Phalanx Biotech (San Diego).

RESULTS

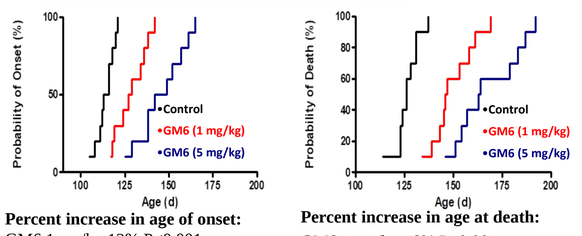
GM6 Neuroprotection in Human CSF

Postmortem CSF from humans with eight different neurological diseases induced rat cortical neurons death when applied as a 10% solution. GM6 provided protection from this injury and increased percentage of cell survival. These studies demonstrate that CSF from humans with neurological disorders contains certain molecules that induce cell death and that GM6 can protect against these effects.



There is statistically significant increase in cell survival (p<0.0001) in ALS (+75%).

GM6 in ALS SOD1 mouse model results

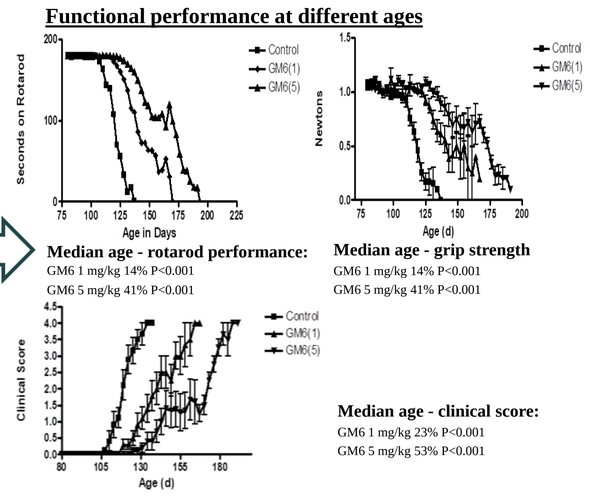


Percent increase in age of onset:
GM6 1 mg/kg 12% P<0.001
GM6 5 mg/kg 27% P<0.001

Percent increase in age at death:
GM6 1 mg/kg 16% P<0.001
GM6 5 mg/kg 30% P<0.001

GM6 improved function in ALS (SOD1-G93A)

Functional performance at different ages



Median age - rotarod performance:
GM6 1 mg/kg 14% P<0.001
GM6 5 mg/kg 41% P<0.001

Median age - grip strength
GM6 1 mg/kg 14% P<0.001
GM6 5 mg/kg 41% P<0.001

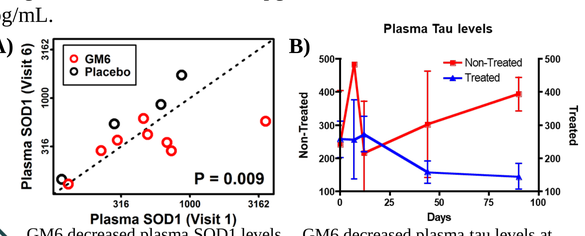
Median age - clinical score:
GM6 1 mg/kg 23% P<0.001
GM6 5 mg/kg 53% P<0.001

Phase 2A Clinical Study

Some GM604-treated patients (2 of 8) exhibited mild rash, but otherwise adverse event frequency was similar in treated and placebo groups. GM604 slowed functional decline (ALSFRS-R) when compared to a historical control (P = 0.005). At one study site, a statistically significant difference between treatment and control groups was found when comparing changes in respiratory function (FVC) between baseline and week 12 (P = 0.027). GM604 decreased plasma levels of key ALS biomarkers relative to the placebo group (TDP-43, P = 0.008; Tau, P = 0.037; SOD1, P = 0.009). We observed favorable shifts in ALS biomarkers and improved functional measures during the Phase 2A study. To confirm these trends, a larger trial for phase 3 is being planned.

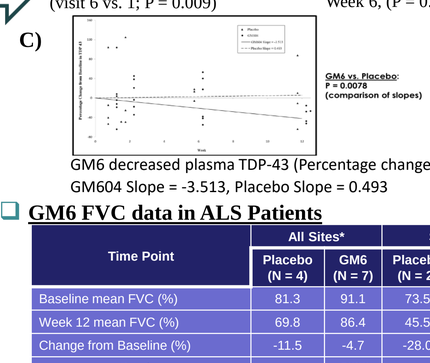
Phase 2A Clinical Study results

GM6 decreased plasma levels of ALS biomarkers: SOD1, P=0.009 (A); Tau, P=0.037 (B); TDP-43, P=0.008 (C). Plasma SOD1 levels in ng/mL. Plasma Tau levels in pg/mL. Plasma TDP-43 levels in pg/mL.



GM6 decreased plasma SOD1 levels (visit 6 vs. 1; P = 0.009)

GM6 decreased plasma tau levels at Week 6, (P = 0.03). Y axis in pg/mL



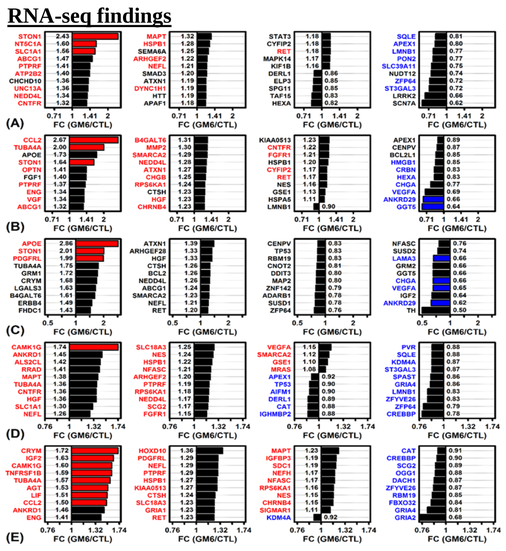
GM6 decreased plasma TDP-43 (Percentage change) at week 12, (P = 0.008)
GM604 Slope = -3.513, Placebo Slope = 0.493

GM6 FVC data in ALS Patients

Time Point	All Sites*		Site 001	
	Placebo (N = 4)	GM6 (N = 7)	Placebo (N = 2)	GM6 (N = 4)
Baseline mean FVC (%)	81.3	91.1	73.5	89.5
Week 12 mean FVC (%)	69.8	86.4	45.5	84.8
Change from Baseline (%)	-11.5	-4.7	-28.0	-4.8
P-value		.5393		.0268

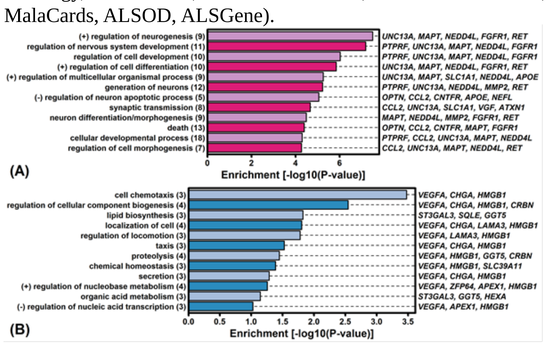
GM6 slowed the decline in Forced Vital Capacity at Site 001

RNA-seq findings



ALS-associated genes most strongly altered by GM6 (RNA-seq).

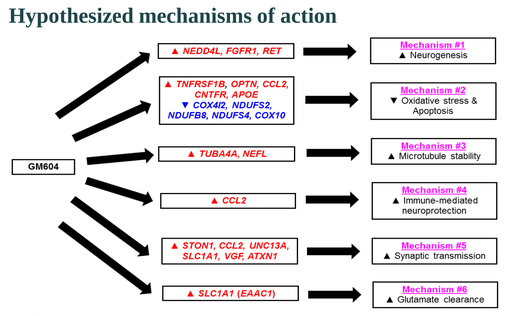
The figure shows ALS-associated genes most strongly altered in SH-5YSY cells (A) treated with GM6 for 6 hours (Experiment 1, see Table 1), (B) treated with GM6 for 24 hours (Experiment 2), (C) treated with GM6 for 48 hours (Experiment 3), (D) treated with GM6 for 6 hours (Experiment 4), and (E) treated with GM6 for 24 hours (Experiment 5). ALS-associated genes with lowest p-values are shown in each case. Genes listed with red or blue font (left margin) were significantly altered by GM6 (FDR < 0.10). Red or blue bars denote genes additionally altered by GM6 based upon a FC threshold (FC > 1.50 or FC < 0.67). Genes were linked to ALS based upon 2 or more of 9 possible database sources (NHGRI-EBI GWAS Catalog, MeSH Database, Disease Ontology, DisGeNET, KEGG Database, eDGAR Database, MalaCards, ALSOD, ALSGene).



Gene ontology biological process (BP) terms associated with ALS-associated genes altered by GM6.

Experiments 1 - 3, Sunny Biodiscovery labs:(A) Gene ontology BP terms enriched with respect to GM6-increased ALS-associated genes (FDR < 0.10; pooled from experiments 1 - 3). Values in parentheses (left margin) indicate the number of GM6-increased genes in each category (right margin: exemplar genes most strongly induced by GM6). (B) Gene ontology BP terms enriched with respect to GM6-decreased ALS-associated genes (FDR < 0.10; pooled from experiments 1 - 3). Values in parentheses (left margin) indicate the number of GM6-decreased genes in each category (right margin: exemplar genes most strongly repressed by GM6).

Hypothesized mechanisms of action



The figure summarizes 6 proposed mechanisms of action for GM6 based upon the identification of ALS-associated genes regulated by GM6 in SH-5YSY cells RNA-seq.

This analysis identified ALS-associated genes regulated by GM6 consistent with 6 potential mechanisms of action.

1. Promotion of neurogenesis: GM6 increased expression of genes known to function in neurogenesis, including *NEDD4L* (neural precursor cell expressed, developmentally down-regulated 4-like, E3 ubiquitin protein ligase), *FGFR1* (fibroblast growth factor receptor 1) and *RET* (ret proto-oncogene).

2. Inhibition of oxidative stress and apoptosis: Expression of *TNFRSF1B* (TNF receptor superfamily member 1B) was increased by 59% following GM6 treatment (Experiments 4 and 5; P = 4.96e-05). Additionally, GM6 increased expression of *OPTN* (optineurin) slightly in all experiments, with a significant 41% increase after 24 hours of stimulation in one RNA-seq experiment (Experiment 2; P = 3.79e-06). Finally, GM6 decreased expression of several genes linked to mitochondria and/or cellular respiration (e.g., *COX4I2*, *NDUFS2*), suggesting that GM6 may attenuate mitochondrial abundance or dysfunction with secondary attenuation of oxidative stress levels.

3. Bolster microtubule stability: Expression of *TUBA4A* was consistently elevated by GM6 in RNA-seq experiments. GM6 also consistently increased expression of *NEFL* (neurofilament light) in all experiments (Experiments 1-5).

4.Immune-mediated neuroprotection: *CCL2* expression was significantly increased by GM6 in both RNA-seq studies (FC > 1.50, P <0.000249).

5.Synaptic transmission: GM6 increased expression of genes influencing endocytosis and/or synaptic vesicle release, including *STON1*, *CCL2*, *UNC13A*, *SLC1A1*, *VGF* and *ATXN1*. Up-regulation of *UNC13A* expression was an early effect of GM6 treatment, with expression increased by 36% following 6 hours of GM6 treatment (P = 2.23e-05), whereas VGF expression was up-regulated later at 24 hours treatment (Experiments 1-3; FC = 1.34; P = 0.000307).

6. Glutamate Clearance: Up-regulation of *SLC1A1* expression was a rapid effect of GM6, with increased expression observed following 6 hours of treatment in both RNA-seq datasets (Experiment 1: FC = 1.56; P = 0.000123; Experiment 4: FC = 1.30; P = 0.000234).

CONCLUSIONS

We have shown that GM6 promotes neuroprotection against toxic factors contained in CSF from patients with neurodegenerative diseases and importantly can cross the blood brain barrier. In the SOD1 mouse model of ALS, we demonstrated that GM6 delays disease onset and reduces mortality, with concomitant improvements in physiological function. Finally, the data in our Phase 2A clinical trial of only 2 weeks (6 doses) suggested a positive signal of GM6 in ALS patients with favorable shifts in disease biomarkers and functional measurements. The data supports further study. A Phase 3 study based on a longer treatment period and larger sample size is now being planned. Based upon our gene expression profiling findings, we have tentatively proposed a mechanism of action (MOA) for GM6 in the setting of ALS. This MOA posits that GM6 protects neurons by combating oxidative damage while bolstering neurogenesis, microtubule stability, immune-mediated neuroprotection, synaptic transmission and glutamate clearance.