Original Investigation

Safety and Clinical Effects of Mesenchymal Stem Cells Secreting Neurotrophic Factor Transplantation in Patients With Amyotrophic Lateral Sclerosis
Results of Phase 1/2 and 2a Clinical Trials

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IMPORTANCE Preclinical studies have shown that neurotrophic growth factors (NTFs) extend the survival of motor neurons in amyotrophic lateral sclerosis (ALS) and that the combined delivery of these neurotrophic factors has a strong synergistic effect. We have developed a culture-based method for inducing mesenchymal stem cells (MSCs) to secrete neurotrophic factors. These MSC-NTF cells have been shown to be protective in several animal models of neurodegenerative diseases.

OBJECTIVE To determine the safety and possible clinical efficacy of autologous MSC-NTF cells transplantation in patients with ALS.

DESIGN, SETTING, AND PARTICIPANTS In these open-label proof-of-concept studies, patients with ALS were enrolled between June 2011 and October 2014 at the Hadassah Medical Center in Jerusalem, Israel. All patients were followed up for 3 months before transplantation and 6 months after transplantation. In the phase 1/2 part of the trial, 6 patients with early-stage ALS were injected intramuscularly (IM) and 6 patients with more advanced disease were transplanted intrathecally (IT). In the second stage, a phase 2a dose-escalating study, 14 patients with early-stage ALS received a combined IM and IT transplantation of autologous MSC-NTF cells.

INTERVENTIONS Patients were administered a single dose of MSC-NTF cells.

MAIN OUTCOMES AND MEASURES The primary end points of the studies were safety and tolerability of this cell therapy. Secondary end points included the effects of the treatment on various clinical parameters, such as the ALS Functional Rating Scale–Revised score and the respiratory function.

RESULTS Among the 12 patients in the phase 1/2 trial and the 14 patients in the phase 2a trial aged 20 and 75 years, the treatment was found to be safe and well tolerated over the study follow-up period. Most of the adverse effects were mild and transient, not including any treatment-related serious adverse event. The rate of progression of the forced vital capacity and of the ALS Functional Rating Scale–Revised score in the IT (or IT+IM)–treated patients was reduced (from −5.1% to −1.2%/month percentage predicted forced vital capacity, $P < .04$ and from −1.2 to 0.6 ALS Functional Rating Scale–Revised points/month, $P = .052$) during the 6 months following MSC-NTF cell transplantation vs the pretreatment period. Of these patients, 13 (87%) were defined as responders to either ALS Functional Rating Scale–Revised or forced vital capacity, having at least 25% improvement at 6 months after treatment in the slope of progression.

CONCLUSIONS AND RELEVANCE The results suggest that IT and IM administration of MSC-NTF cells in patients with ALS is safe and provide indications of possible clinical benefits, to be confirmed in upcoming clinical trials.

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The biological mechanisms underlying amyotrophic lateral sclerosis (ALS) are only partially understood, and there is currently no effective treatment that can significantly halt or reverse disease progression. Although neurotrophic factors (NTFs) have been shown to extend the survival of motor neurons in ALS, peripheral administration of single NTFs has not revealed any benefit.[1,2] A more effective approach might be direct delivery of multiple NTFs to the central nervous system through transplantation of cells that actively secrete these factors. Indeed, studies in animal models of neurodegenerative diseases have shown that NTF-secreting cells induce neuroprotective effects.[3-7]

Systemic or intraspinal bone marrow or adipose-derived mesenchymal stem cell (MSC) transplantation has been reported to delay motor neuron degeneration and improve motor performance in the mouse model of ALS.[8-10] The benefits of MSC treatment were attributed to significant upregulation of various NTFs, especially the glial-derived neurotrophic factor (GDNF) and basic fibroblast growth factor.[8] In parallel, immunomodulation and increased neurogenesis were suggested as additional mechanisms for these favorable effects.[11,12] The outcome of pilot clinical studies of MSC therapy suggests some neurological stabilization in patients with ALS[13-16] and possible neuroregeneration of the optic tracts in progressive multiple sclerosis[17] and in multiple system atrophy.[18]

Stem cell therapy has been pursued by us and others in various neurological diseases as a novel means of promoting regeneration and neuroprotection.[13-19] Mesenchymal stem cells have become the most common type of adult stem cells used in clinical trials owing to their safety profile and their immunomodulatory and neuroprotective effects.[20,21]

Using a medium-based differentiation process, we have induced MSCs to become MSC-NTF cells, with markedly enhanced secretion of NTFs such as GDNF, brain-derived NTF, vascular endothelial growth factor (VEGF), and hepatocyte growth factor.[4-7] The protective effects of MSC-NTF cells in animal models of neurodegenerative diseases, and the encouraging results of compassionate treatments, supplied the rationale for investigating autologous MSC-NTF cell transplantation in patients with ALS.

We conducted a phase 1/2 study of a single intramuscular (IM) or intrathecal (IT) administration of autologous MSC-NTF cells. Following a positive interim safety analysis performed after completion of 12 of the targeted 24 patients, the trial was transformed to a phase 2a dose-escalating study, by combined IM and IT administration of the MSC-NTF cells. The studies shared key design elements, including visit schedule and activities, efficacy end points, and the major inclusion and exclusion criteria, thus allowing their analysis as 1 study.

Methods

Study Design

Both parts of the trial were single-arm, open-label, proof-of-concept studies that featured a 3-month run-in period, administration of cells, and a 6-month follow-up period conducted at the Hadassah Medical Center, Jerusalem, Israel. The

Key Points

Question: What are the safety and clinical effects of treatment with mesenchymal stem cells induced to secrete neurotrophic factors (MSC-NTF cells) in patients with amyotrophic lateral sclerosis (ALS)?

Findings: In 2 consecutive open-label studies, treatment of patients with ALS with autologous MSC-NTF cells by intrathecal (IT), intramuscular (IM), or combined (IT+IM) administration was found to be safe and well tolerated. The rate of progression of forced vital capacity and ALS Functional Rating Scale–Revised score in the IT (or IT+IM)–treated patients was reduced.

Meaning: Intrathecal and IM administration of MSC-NTF cells in patients with ALS is safe with indications of possible, clinically meaningful benefits.

2 stages of the trial were approved by the local ethics committee of the Hadassah Medical Center and by the National Ethics Committee of the Israel Ministry of Health, conducted in accordance with the Declaration of Helsinki and registered in ClinicalTrials.gov (NCT01051882 and NCT01777646). The patients provided written informed consent.

Participants

For both stages of our trial, eligible participants were between 20 and 75 years of age, fulfilled the El-Escorial criteria[22] for definite or probable ALS, and had a history of ALS of less than 2 years' duration. Between June 2011 and November 2012, 12 patients were enrolled in the phase 1/2 part of the study. Of these, 6 patients with baseline ALS Functional Rating Scale–Revised (ALS-FRS-R) scores of more than 30 received cells IM and 6 patients with more advanced disease (ALS-FRS-R score <30 and >15 and forced vital capacity [FVC] >50%) were treated IT. A planned interim safety analysis was performed after treated patients completed the study protocol. This resulted in the conversion of the study to a dose-escalating phase 2a study. In the phase 2a part of the trial, 14 patients with baseline ALS-FRS-R score greater than 30 and baseline FVC at least 50% were enrolled between December 2012 and October 2014 (Figure 1).

Procedures

The visit schedule consisted of a 3-month run-in period that included a screening visit, an enrollment visit, and bone marrow aspiration and transplantation followed by follow-up visits every 6 months after transplantation. Bone marrow was aspirated 2 months after the screening visit, and clinical-grade MSC-NTF cells were manufactured by a current good manufacturing practice–compliant process in the clean room facility at the Hadassah Medical Center (Jerusalem, Israel) by BrainStorm Cell Therapeutics. Mesenchymal stem cells were isolated from the patients' bone marrow, expanded ex vivo, and induced to differentiate into MSC-NTF cells, using a medium containing 1mM of dibutyrylcyclic adenosine monophosphate, 20 ng/mL of human basic fibroblast growth factor, 5 ng/mL of human platelet-derived growth factor, and 50 ng/mL of human heregulin β1. The cells were used for transplantation when they

Figure 1. Flowcharts of the Phase 1/2 and Phase 2a Studies

Phase 1/2

Phase 2a

IM indicates intramuscular;
IT, intrathecal.
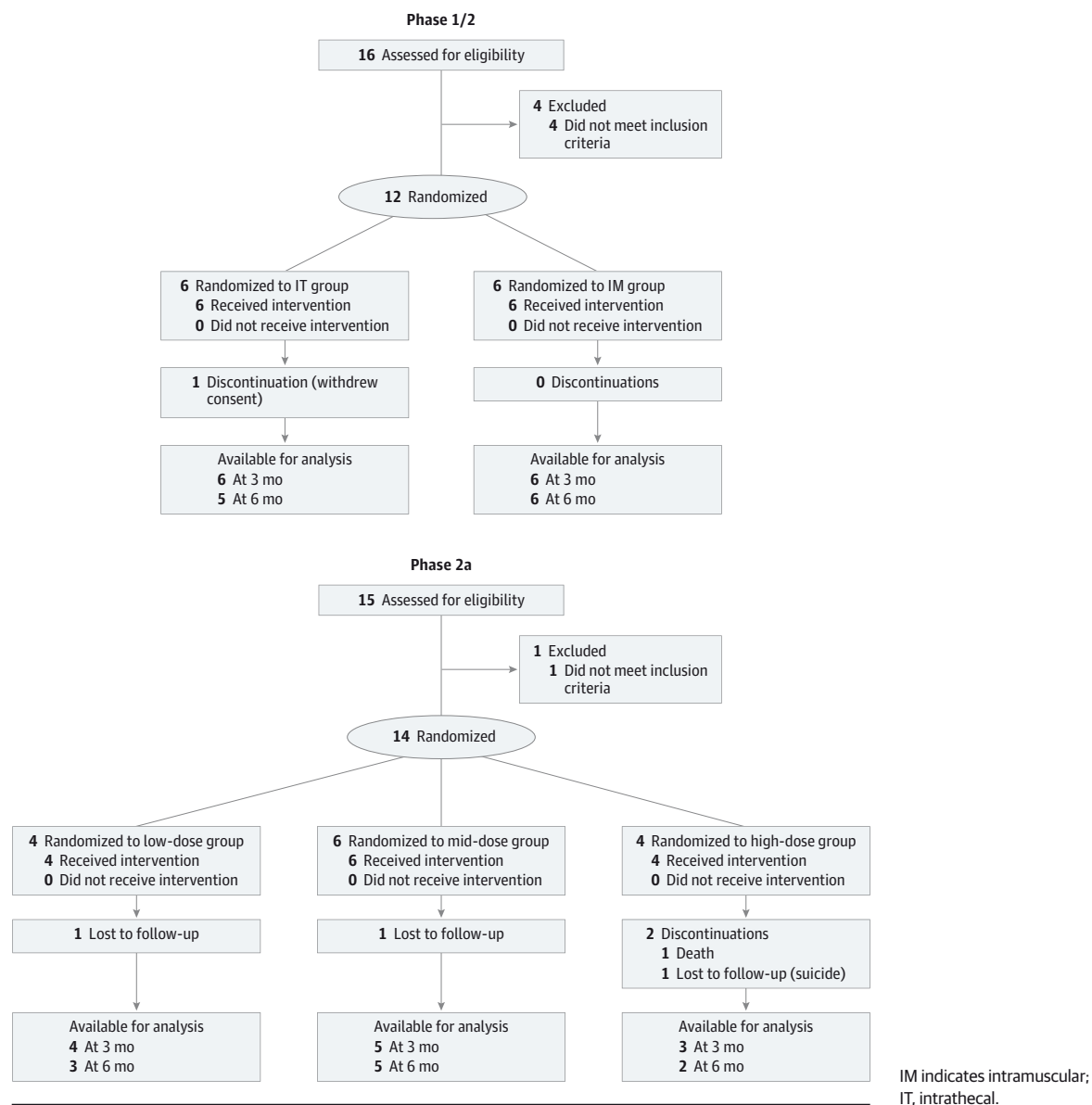
complied with the release test specifications, which included safety, potency (NTF secretion), and identity (surface marker characterization).

In the first part (phase 1/2) of the trial, the MSC-NTF cells were administered by IM injections at 24 separate sites to the biceps and triceps (1×10^6 cells/site) or by IT administration of 1×10^6/kg cells. In the second stage (phase 2a) of the study, the patients were treated both IT and IM in 3 dosing cohorts (low dose: 1×10^6 cells/kg IT and 24×10^6 cells IM; mid-dose: 1.5×10^6 cells/kg IT and 36×10^6 cells IM; and high dose: 2×10^6 cells/kg IT and 48×10^6 cells IM). Following administration of the MSC-NTF cells, the patients remained hospitalized for up to 72 hours for observation of possible adverse events.

Peripheral blood samples obtained at visits 1, 4, 5, 7, and 10 were examined for various standard safety biochemical and

blood count parameters. Blood mononuclear cells were tested by flow cytometry for the expression of several surface lymphocyte markers.

Muscle volume was determined at visits 1, 4, 5, 7 (only in the second part of the study), and 10 by computerized analysis of magnetic resonance imaging (MRI) scans of the left and right arms with ScanIP 2012 Simpleware Ltd Software, using the T1-weighted transversal sequence. The available scans for each patient were analyzed and the resulting muscle volumes were given in milliliters.

Compound muscle action potentials (CMAPs) were recorded at monthly intervals from the bicep muscle. The electrodes were fixed at the mid-distance between the elbow and the shoulder (active) and at the elbow. Stimulation of the musculocutaneous nerve was applied at the Erb point. For each

Table 1. Summary of Adverse Events

Adverse Event	No.		
	Phase 1/2 (n = 12)	Phase 2a (n = 14)	Total (n = 26)
Headache	2	11	13
Fever	3	8	11
Back/leg pains	0	8	8
Vomiting	0	3	3
Neck stiffness	1	1	2
General weakness	0	1	1
Bruising	1	0	1
Spasticity	0	1	1

patient, localization of the electrode was measured and recorded at the first visit and repeated thereafter. The CMAP amplitude was measured from peak to peak in millivolts.

Outcomes

The primary end points of the investigation were the safety and tolerability of a single IM or IT administration (phase 1/2) or of combined IT and IM delivery (phase 2a). Secondary efficacy end points included the rate of change in the ALS-FRS-R score, obtained by an independent certified neurologist; FVC[21,22]; and changes in muscle mass (estimated by 3-dimensional MRI) and in CMAPs during the 6-month posttreatment period compared with those in the run-in period.

Statistical Analysis

Parameters measured as changes over time were calculated as simple linear regressions to compare the preintervention and postintervention slopes of the ALS-FRS-R gradients and the FVC. For the post hoc analysis, all patients who received IT administration in the 2 studies, a piecewise linear model with a random patient effect was used to compare the preintervention and postintervention gradients of ALS-FRS-R score and FVC and to test for statistical significance.

Results

The eTable in the Supplement summarizes the participants' demographic characteristics. Eleven of the 12 patients in the phase 1/2 part of the study and 10 of 14 patients in the phase 2a stage completed all follow-up visits. No serious adverse events were associated with IT, IM, or IT+IM administration of MSC-NTF cells. In the phase 1/2 part, there was a single serious adverse event (nephrolithiasis, unrelated to treatment). A patient in the IT cohort withdrew consent after visit 7 and later died of pneumonia related to disease progression. In the phase 2a part of the trial, there were 2 deaths, which were considered unrelated to the treatment: 1 involved a physician-assisted suicide and 1 was due to a clearly defined (and unrelated-to-treatment) pathology (deteriorating chronic hyponatremia that resulted in cardiac complications).

Most of the observed adverse events were of grades I and II and transient, appearing in close association with the administration of MSC-NTF cells, lasting a few hours or up to 3 days following transplantation (Table 1). These included mainly headache, fever, vomiting, leg and back pain, and neck stiffness. There were no significant changes in any laboratory parameters, including blood counts, biochemistry, renal and hepatic function, thyroid function, and urinalysis. Magnetic resonance images of the arm muscles did not reveal any significant pathology at the site of injection (neither infection nor tumor formation) in any patient.

Neurotrophic growth factor secretion of the patients' cells in the 2 studies was shown to be induced in the MSC-NTF cells compared with the MSCs of the same patient prior to differentiation (eFigure 1 in the Supplement). Despite the differences in specific productivity, which are the results of patient-to-patient variability, in all samples tested, MSC-NTF cells secreted significantly more NTFs compared with the MSCs of the same patient.

The rate of change in ALS-FRS-R score and FVC (percentage predicted) during the 6-month period following administration of the MSC-NTF cells was compared with that of the 3-month run-in period. In the phase 1/2 part of the study, there was an improvement in the mean monthly rate of progression of the ALS-FRS-R score and FVC in the IT cohort (from −1.56 to 0.28 and from −3.5% to −2.3%, respectively), but not in the IM-treated group (Figure 2A and Figure 3A). In the phase 2a stage of the trial, a more substantial improvement in the monthly rate of decline of ALS-FRS-R score and FVC was observed following MSC-NTF cell transplantation (from −1.4 to −0.6 and −2.6% to 0.86%, respectively; Figure 2B and Figure 3B). No clear dose effects were detected.

Because only IT-treated patients from both studies appeared to experience systemic benefit, a post hoc comparison was made between the progression rate of ALS-FRS-R score and of FVC during the pretreatment vs the posttreatment periods for patients from both studies with 6 months of follow-up who were treated IT or IT+IM, using a piecewise linear regression analysis with a patient random effect. This analysis revealed a statistically significant improvement in the rate of FVC progression (P = .036; n = 15) and a trend (very close to statistical significance) of improvement in the rate of ALS-FRS-R score progression (P = .052; n = 15) for those with complete follow-up.

An additional post hoc per-patient analysis of the rate of disease progression in the same combined subgroup of patients (treated IT or IT+IM) was also performed. Patients who demonstrated a posttreatment improvement in the slope of at least 25%, to either ALS-FRS-R score or FVC compared with the run-in period, were considered responders. Of those with 3 months of follow-up (n = 18), 78% (n = 14) were responders, and of those with 6 months of follow-up (n = 15), 87% (n = 13) were responders (Table 2). At 6 months after treatment, 80% (n = 12) of the patients improved by more than 35% and 67% (n = 10) by more than 50% to either ALS-FRS-R score or FVC (Table 2).

In the phase 1/2 part of the study, an (expected) decline in CMAPs during the run-in period was observed, which improved after transplantation, especially in the IM-injected patients and in the right (injected) arm (eFigure 2A in the Supplement). In the phase 2a stage, there was a similar indication of

Figure 2. Longitudinal Follow-up of the Changes in the Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised (ALS-FRS-R) Score

A ALS-FRS-R scores in phase 1/2

B ALS-FRS-R scores in phase 2a
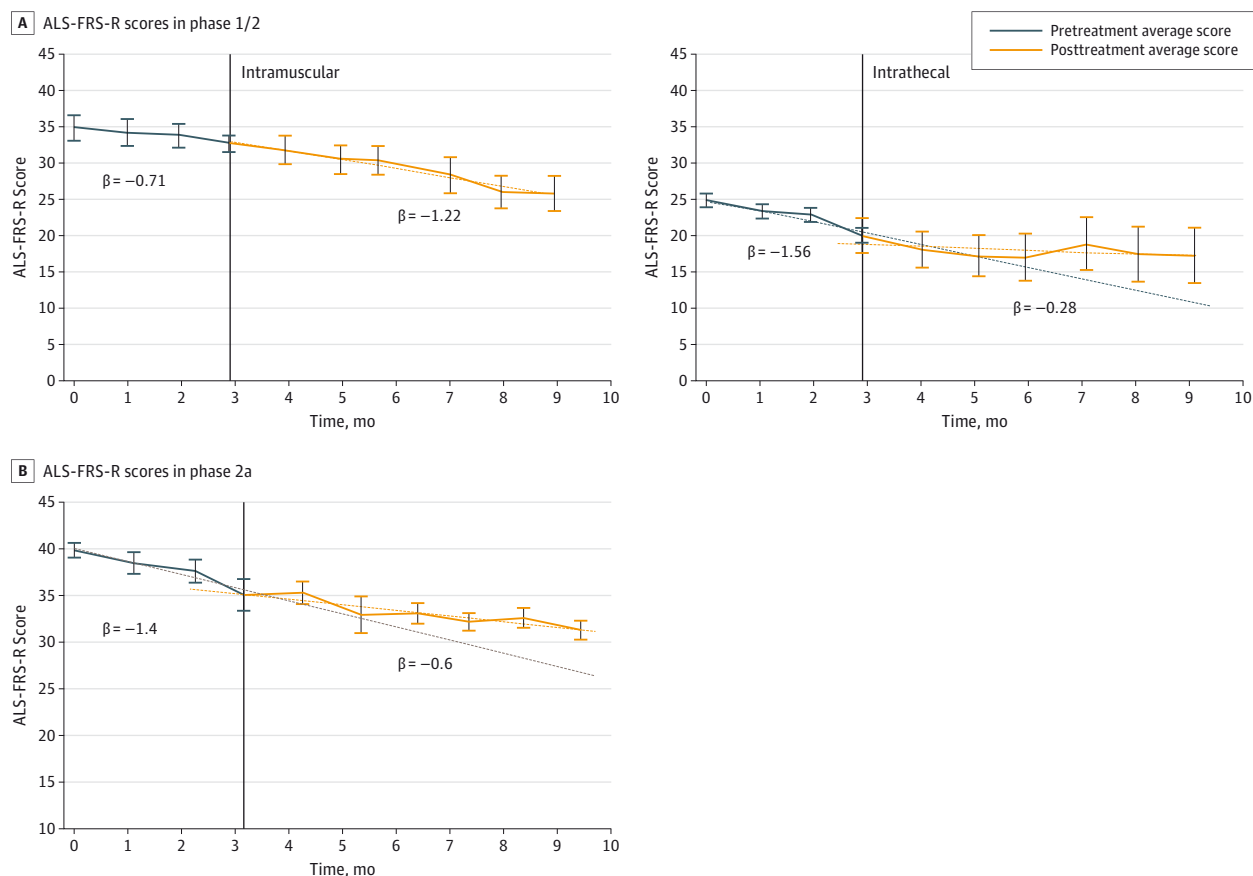
The mean (SEM) of the ALS-FRS-R score of patients with ALS treated intramuscularly or intrathecally in the phase 1/2 study (A) and by combined intrathecal and intramuscular administration in the phase 2a study (B).

The vertical black lines indicate the times of treatment; the dashed lines, the slopes (β) for pretreatment and posttreatment.

a beneficial effect favoring the injected (right) arm (eFigure 2B in the Supplement). Neither were statistically significant.

As expected, muscle atrophy progression was documented by MRI of the arm muscles in both parts of the trial. In the first part of the trial, the data obtained were not sufficient for meaningful comparisons owing to the lack of an adequate number of quality MRI scans for analysis, and the fact that the posttreatment scan was performed at day 1 after transplantation (to detect possible local adverse effects) and was greatly influenced by the changes in the degree of edema at the site of injection. In the 2a trial, a difference in the progression of muscle volume loss between the right (injected) and the left (noninjected) arm was observed (eFigure 3 in the Supplement). These findings are in agreement with the CMAP data and indicate a trend toward a local effect of the transplantation in the right arm.

The patients treated with MSC-NTF cells in our trial demonstrated some systemic immunologic response to MSC-NTF cell treatment. Patients in both parts of the study showed a trend toward upregulation of the regulatory T-lymphocytes (CD4+CD25+) for up to 3 months following transplantation (eFigure 4 in the Supplement).

Discussion

The current clinical trial consisting of 2 parts, a first-in-man phase 1/2 study and a phase 2a dose-escalation stage, shows that IT and IM injection of MSC-NTF cells in patients with ALS is safe and well tolerated over the study follow-up period. Adverse events that were considered related to the treatment were mostly mild and transient and occurred close to the time of cell administration. These findings match previous observations of treatment with MSCs in a variety of diseases, including ALS.[13-19] Although our study was primarily targeted to assess safety and not powered for efficacy, the data also provided some indications of clinically meaningful beneficial effects induced by the IT treatment with MSC-NTF cells. These are reflected by the slower rate of disease progression (assessed by the changes in ALS-FRS-R score and FVC) during the 6 months following transplantation vs the 3-month run-in period in this patient subgroup. On the basis of an individualized per patient analysis, most of the IT- or IT+IM–transplanted patients (89% at 3 months [n = 16] and 87% at 6 months [n = 13]) were defined as responders (having a slower progression rate after

Figure 3. Longitudinal Follow-up of the Changes in the Forced Vital Capacity Score

A Forced vital capacity in phase 1/2

B Forced vital capacity in phase 2a
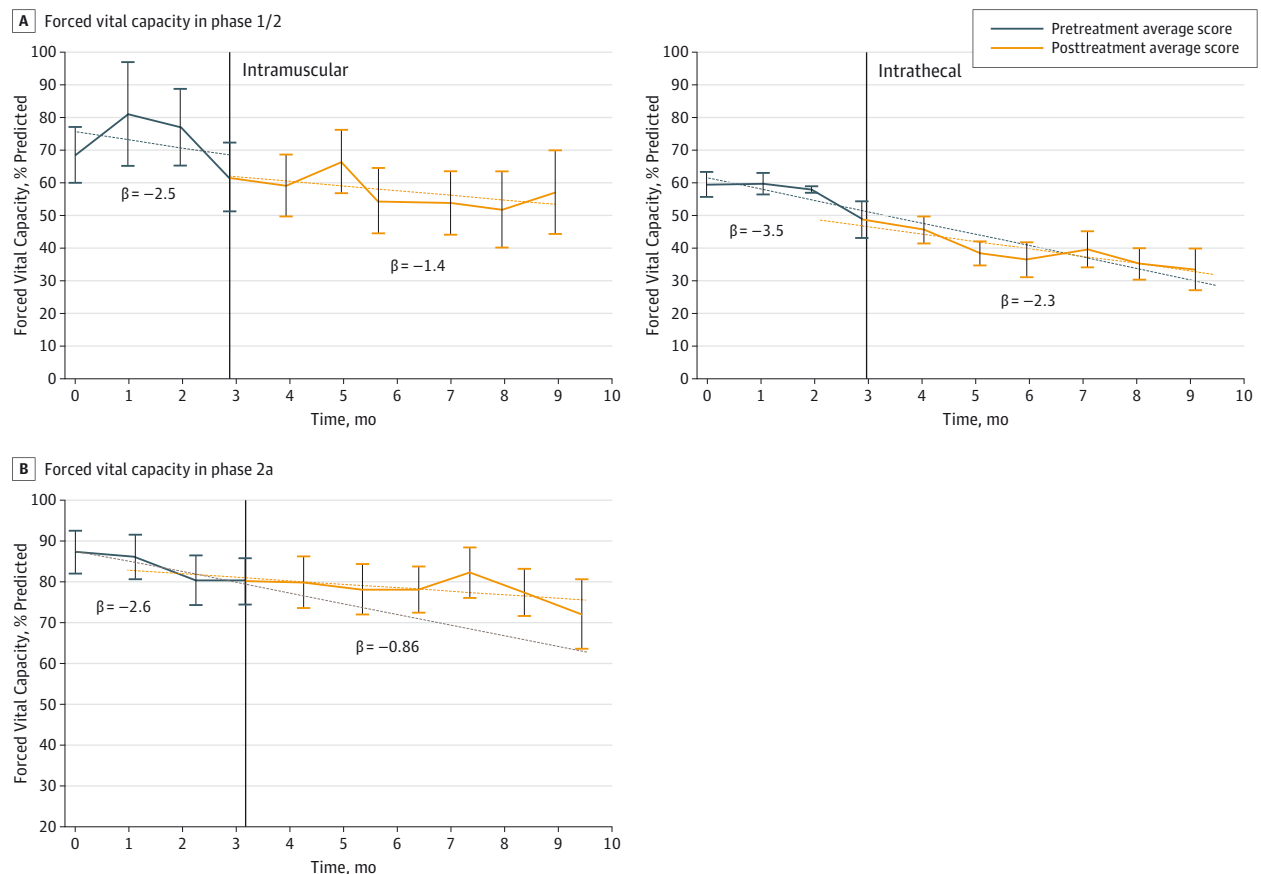
Mean forced vital capacity (percentage predicted) ± SEM score of patients with amyotrophic lateral sclerosis in the phase 1/2 study treated intramuscularly or intrathecally (A) and by combined intrathecal and intramuscular administration in the phase 2a study (B). The vertical black lines indicate the times of treatment; the dashed lines, the slopes (β) for pretreatment and posttreatment.

Table 2. Patients Responding to the Treatment in the Subgroup of IT or IT+IM–Treated Individuals From Both Trials[a]

Group	3 mo			6 mo		
	ALS-FRS-R	FVC	ALS-FRS-R or FVC	ALS-FRS-R	FVC	ALS-FRS-R or FVC
Phase 1/2	5 of 6	3 of 6	5 of 6	4 of 5	3 of 5	3 of 5
Phase 2a	6 of 12	8 of 12	11 of 12	5 of 10	6 of 10	8 of 10
Total No. of patients	18			15		
No. of patients showing improvement	11	11	16 (3 of which $P < .10$)	9	9	13 (7 of which $P < .10$)
Patients showing any degree of improvement, %	61	61	89	60	60	87
Patients showing >25% improvement, %	56	50	78	50	60	87
Patients showing >35% improvement, %	56	45	72	53	60	80
Patients showing >50% improvement, %	39	44	61	40	53	67

Abbreviations: ALS-FRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised; FVC, forced vital capacity; IM, intramuscular; IT, intrathecal.

[a] Patients were defined as responders to the treatment if they had less monthly progression in ALS-FRS-R score or FVC at 3 or 6 months following transplantation than during the 3-month pretreatment run-in period.

treatment compared with the pretreatment run-in period) of ALS-FRS-R score or FVC. A trend toward a beneficial effect was also observed in terms of 2 novel biomarkers, the rate of decline of muscle volume and of the CMAPs, that were most prominent in the right (injected) arm and in the IM-treated cohort (eFigures 2 and 3 in the Supplement), possibly indicating a localized neurotrophic effect at the site of transplantation. To our knowledge, this is the first human experience with

stem cells that have been induced under culture conditions to produce NTFs, thus bearing the potential to support neuronal survival (neurotrophic/neuroprotective effect) and to modify the course of neurodegeneration in ALS.

The current trial is novel in 3 aspects. First, a new type of autologous MSCs (MSC-NTF cells), developed using an innovative medium-based technique to induce the secretion of NTFs and thus increase their neuroprotective potential,[3-7] was applied clinically for the first time. Second, 2 unique routes of administration were used: a central one (directly to the cerebrospinal fluid to circumvent the blood-brain barrier) and a peripheral one into the muscles. Third, additional biomarkers were used for the quantitative measurement of progression of neurodegeneration and muscle atrophy: calculation of muscle volume using 3-dimensional MRI and measurement of the CMAP amplitude of the same muscles.

Stem cell–based therapies have been proposed as potential treatments for a variety of neurodegenerative diseases. Small clinical trials using adult stem cells in a number of neurological diseases have depicted the safety of such treatments.[13-19] In the MSC studies, immunomodulatory mechanisms have been suggested to be central to the reported beneficial effects.[19] Specifically for ALS, pilot trials using IT, intravenous and intraspinal injection of MSCs, have shown the safety of the procedure paralleled by some weak indications of clinical benefits.[13-16] It is possible that the beneficial clinical trends observed in our study may be attributed to the improved capacity of the MSC-NTF cells to secrete NTFs compared with naive MSCs, thus exerting—at least theoretically—a more potent neuroprotective effect.

An additional explanation may be related to the immunomodulatory effects of MSC-NTF cells on central nervous system microglial cells, especially in light of the observed upregulation of the regulatory cells in our study. Activation of microglial cells results in the production of proinflammatory mediators that are toxic to neurons and was suggested to contribute to the pathology of ALS.[23-26]

Several studies have shown that MSCs inhibit microglial activation and are capable of reprogramming microglial cells into an M2-like phenotype characterized by increased phagocytic activity and upregulated expression of anti-inflammatory mediators.[27,28]

Additionally, the mode of administration of MSC-NTF cells in our studies (especially the IT route) may be also crucial for the observed therapeutic effect. Intrathecal transplantation, as previously advocated and applied by our group[14,29] brings the cells in closer proximity to extensive areas of the central nervous system and in particular to the lower motor neuron cell bodies. Prior clinical trials with single NTFs in ALS relied on peripheral delivery.[1,2] Given the very short half-life of these growth factors when administered peripherally, the suboptimal clinical effects in those trials do not necessarily rule out the potential therapeutic benefits of NTFs in ALS.[30] Thus, in our study, we were able to safely deliver a cellular source of NTFs to the subarachnoid space. Indeed, IT administration of MSCs previously showed neuroprotective effects in animal studies and in clinical trials.[31,32] The rationale for IM administration of MSC-NTF cells was based on the notion that initial stages of ALS involve degeneration of nerve terminals in the neuromuscular junction area, and the "dying-back phenomenon."[33] Muscle-derived transgene GDNF significantly delayed the onset of disease and increased the life span of G93A-*SOD1* mice.[34] Intramuscular transplantation of MSCs engineered to secrete GDNF or VEGF was shown to synergistically improve the clinical course of ALS in an animal model and ameliorate motor neuron loss.[35] We previously reported that IM transplantation of muscle progenitor cells, which overexpress NTFs, increases the lifespan in *SOD1* mice.[36] Nevertheless, our current results showed that IM administration induced only a minor local effect in patients with ALS.

The limitations of these open-label studies were the small number of patients and the lack of a control placebo-treated arm.

Conclusions

The possible clinical benefits of our cell therapy may be explained by neuroprotective effects that resulted in improvement in the progression rate of ALS-FRS-R score and FVC. The observed posttransplantation progress rates differ from the expected rate of progression reported in natural course epidemiological studies (of approximately 1 point in the ALS-FRS-R per month)[37]; such degree of average progression rate was observed in our patients during the 3-month run-in pretreatment period. Rapid progression rates (of >0.5 ALS-FRS-R points monthly) have been reported as bad long-term prognostic indicators,[38] suggesting that our cohort consisted of patients with rather aggressive disease. According to the survey conducted by Castrillo-Viguera et al,[37] neurologists who treat patients with ALS believe that a 25% or more reduction in ALS-FRS-R slope is of clinical significance.[37] Therefore, the importance of the observed modification of disease progression rate following MSC-NTF cell therapy in our pilot trials may represent an indication of a clinically meaningful effect, pending further confirmation from the ongoing, double-blind placebo-controlled multicenter phase 2 clinical trial (clinicaltrials.gov Identifier: NCT02017912).

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