Original Research

A phase II clinical trial with repeated intrathecal injections of autologous mesenchymal stem cells in patients with amyotrophic lateral sclerosis

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1. Abstract

Background: Mesenchymal stem cells (MSC) were shown to induce beneficial effects in animal models of neurodegeneration and in pilot human trials in multiple sclerosis and amyotrophic lateral sclerosis (ALS). Aim: An open-label, clinical trial to evaluate the safety and efficacy of repeated intrathecal administrations of autologous MSC in ALS-patients. Methods: The study included 20 subjects (age: 20–70) with definite diagnosis of ALS and Amyotrophic Lateral Sclerosis Functional Rating Scale Revised (ALSFRS-R) score of >20. The patients were treated with 1–4 intrathecal injections of MSC, at intervals of 3–6 months. The primary endpoints were safety and tolerability. Efficacy measurements including ALSFRS-R score and forced vital capacity (FVC), were assessed as secondary endpoints. Results: No serious adverse events were observed during the whole period of the trial. One patient withdrew from the study before the first injection. The monthly rate of progression in ALSFRS-R was ameliorated by more than 25% in 15/19 patients between the 1st and 2nd injection (mean improvement of 107.1%); in 11/12 between the 2nd and 3rd injection and in 8/10 between the 3rd and 4th injection. Overall, during the whole period till the last transplantation 13 patients had a >25% improvement in the slope of progression of ALSFRS-R (mean improvement of 47.4%, p < 0.0038, Wilcoxon rank signed test). 7 out of 19 patients actually improved clinically (range of increase in ALSFRS-R: +1 to +4 degrees) after the first transplantation and 5 remained improved after the second cycle. The response rate correlated with the time-intervals between the injections. Conclusion: The results of our study show that repeated intrathecal injections of autologous MSC was safe in patients with ALS and provide indications of medium-term clinical benefits that were related to the intervals between the administrations of the cells. Larger studies are needed to confirm these observations.
2. Introduction

Amyotrophic lateral sclerosis (ALS) is a degenerative disease affecting the motor neurons in brain and spinal cord with a rapidly progressive course, leading to generalized muscle paralysis and death, usually within 3–5 years from onset. The pathogenesis of ALS is complicated and still poorly understood. 10% of cases are familial and 90% sporadic. No therapy has shown sufficient efficacy in halting the progression of the disease. The two FDA approved therapies, Riluzole and Edravarone showed a mild to moderate effect in terms of reduction of the rate of progression and prolongation of survival by few months [1, 2].

Stem cells in general, were shown to induce neuroprotection and immunomodulation, by secreting growth factors and immunomodulatory cytokines, and by increasing the regulatory T cells [3–10]. Mesenchymal stem cells (MSC) are non-hematopoietic stromal cells, which can be obtained from various sources, i.e., bone marrow (BM-MSC), adipose tissue (AT-MSC), embryonic tissue (E-MSC), cord blood (CB-MSC), reprogramming of mature cells (iMSC) and peri-natal tissue-Wharton’s jelly (WJ-MSC) [11] and amniotic membrane [12].

Their classical role is to support hematopoiesis and produce cells of the mesodermal lineage [13, 14]. Additional properties of the MSC, including immunomodulatory and neurotrophic effects, have been described [4–7, 15–20]. In animal studies, intravenous (IV) and intrathecal (IT) administration of MSC has been shown to suppress experimental autoimmune encephalomyelitis (EAE) [7, 21, 22] and support remyelination following spinal trauma or induced demyelination [23–25]. In the mouse model of ALS, MSC administered intravenously (IV), intrathetically (IT) or intraspinally, improved motor performance and extended the survival of the animals [26–32].

Few small, mostly open-label, clinical trials have reported indications of favorable effects of MSC treatment in neurological diseases, such as stroke, multi-system atrophy, multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS) [33–50]. Specifically, in ALS, the first phase I/II trial from our group, in which 19 patients received both intrathecal and intravenous injections of MSC, showed that this combined administration was safe, in the short term follow up and induced a trend for stabilization of disease progression by at least 25% the progression rate of the disease, especially in the intrathecal treated group [41]. The same type of modified MSC was used in a phase II, randomized, placebo-controlled trial, in which although the rate of disease progression (ALSFRS-R slope change) did not differ between the MSC-NTF and placebo-treated patients, an improvement in the progression rate was noted in a prespecified rapid progressor subgroup, paralleled by an increase of neurotrophic factors and decrease of inflammatory biomarkers in the CSF [51]. Other groups showed in open pilot trials, similar results in terms of safety and indications of efficacy after intrathecal injections of MSC, obtained from various sources [50, 52, 53].

An additional way of MSC-administration through a direct intraspinal injection of the cells, was also tested in phase I and phase IIa small studies in ALS, and showed that the procedure was rather safe and induced short-term clinical improvements in a number of patients [54–57]. Intraspinal administration of neural stem cells showed similar to MSC results, in terms of safety and short-term clinical improvements [58–62].

In most of the above studies the observed beneficial effects were rather short-living, possibly indicating the need of repeated injections. To this direction, a Korean group performed a phase I and a phase II trial using two IT injections of MSC, 26 days apart [48, 63]. In the phase II trial from this group, which was the first randomized controlled study, 64 patients were randomized in 2 groups and received either Riluzole only or Rizulole and two IT injections of MSC; a significant reduction in the rate of progression of the ALS-FRS scores was noted in the MSC-treated group [63]. A larger phase III trial with three bimonthly IT injections of MSC-NTF (Nurown cells, Brainstorm®) or placebo, was recently completed and the final results from it, are pending.

Additional support to possible clinical neuroprotective effects of MSC-treatment was obtained from a recently completed, controlled double-blind phase II study from our group, that examined the effects of MSC transplantation, comparing the intravenous with the intrathecal way of administration, in 48 patients with active progressive multiple sclerosis (MS). In this study [64] during the one-year follow up, 58.6% and 40.6% of patients treated with MSC-IT and MSC-IV, respectively, exhibited no evidence of disease activity (NEDA) compared with 9.7% in the sham-treated group. The intrathecal transplantation induced additional benefits, on relapse rate, MRI and fMRI measurements, walking ability and cognitive tests, possibly indicating that it is superior to the intravenous way of cell-delivery.

The observation that in most of the previously mentioned studies, both in MS and ALS [2, 41, 51, 58, 59, 65, 66], there were signs of fading-off of the beneficial effects by time, prompt us to evaluate the effect of repeated/multiple intrathecal injections of MSC in ALS.
3. Materials and methods

3.1 Patients, study design and treatment protocol

This is a single center open, phase II clinical trial, evaluating the safety and the clinical effects of repeated IT injections of MSC in ALS patients. Patients received 1–4 intrathecal injections of autologous MSC, at intervals of 3–6 months. The scheduled treatment protocol was intended to include IT injections every 3 months for up to 2 years. However, due to limitations in the number of cultured cells or the unwillingness of the patients to undergo repeated lumbar punctures and additional bone marrow harvesting, the treatment intervals were modified/extended in several of the patients to a maximum of 6 months. The study was conducted at the Department of Neurology & Unit of Neuromuscular and Cell therapies, at the Hadassah Hebrew University Medical Center, Jerusalem, Israel under license from the hospital’s Ethics committee and the Israel Ministry of Health, from 2016 to 2019.

The number of the patients recruited (n = 20) was calculated based on an assumption of efficacy of at least 50%, in reduction of the ALSFRS-R monthly progression rate, compared to the rate of progression during the run-in period. We based our calculations both on published cohorts (PRO-ACT, [67, 68]) that showed a mean monthly change ranging from 0.59 to 1.2 in ALSFRS-R and on our previous cohort, in which there was a higher monthly ALSFRS-R change of −1.4 [41]. Various models were evaluated with assumptions of efficacy ranging from 50% to 75%, based on our previous experience with MSC-treatments, indicating that the minimal size of the experimental group should be of at least 20 patients. 250 applications from patients were received in our centre. An independent selective committee was set by hospital’s administration and applications were examined anonymously, according to the predefined inclusion and exclusion criteria (see Fig. 1: Flowchart of the trial). Following inclusion and screening visit, patients were followed up for a mean «run-in» period of 7.8 months (range: 3.5–18) before the first visit (visit 1), to evaluate the progression rate of their disease and up to for 6 months following the treatment intervals were modified/extended in several of the patients to a maximum of 6 months. The study was conducted at the Department of Neurology & Unit of Neuromuscular and Cell therapies, at the Hadassah Hebrew University Medical Center, Jerusalem, Israel under license from the hospital’s Ethics committee and the Israel Ministry of Health, from 2016 to 2019.

One month later, patients underwent bone marrow aspiration (BMA) and MSC cells were produced from the bone-marrow aspirated. On the treatment visit, the patients were transplanted with an IT injection of MSC (1 × 10^6 cells per kg of body weight) and thereafter with additional injections every 3–6 months.

Patients had bimonthly follow up after the treatment, which included observation for side effects, full neurological evaluation and muscle chart, ALSFRS-R score and forced vital capacity (FVC) test. Safety was assessed following treatment by the MSC, using measurements of the following variables: physical examination, vital signs (heart rate, blood pressure, body temperature), and laboratory parameters: WBC with differential and platelet count, hemoglobin (Hb), hematocrit (Ht), blood chemistry for electrolytes, creatinine and liver enzymes.

3.1.1 Inclusion criteria

- Fulfillment of the El-Escorial criteria for definite ALS (sporadic and not familial).
- Male or female gender and age range between 20 and 70 years.
- ALS-FRS-R score of at least 20 and disease duration of less than 3 years.
- Understanding of the nature of the procedure and signature of a written informed consent prior to any study procedure.

3.1.2 Exclusion criteria

- A positive test for HBV, HCV or HIV.
- High protein concentration in the CSF (Protein >60 mg/mL).
- Lymphocytosis in the CSF (lymphocytes >10/mL).
- Positivity for anti-GM1 antibodies.
- Evidence of significant conduction blocks or slow nerve conduction velocities (a reduction of more than 30%) confirmed by nerve conduction velocity/EMG studies.
- Respiratory dependendency.
- Impaired hepatic function (ALT, AST or GGT 2-fold higher than normal upper limit).
- History of significant cardiac disease, malignant disease or any other disease that may risk the patient or interfere with the ability to interpret the results.
- Active infections.
- Participation in other clinical trial within 6 month prior to start of the current study.
- Previous treatment with any cellular therapy.
- Unwillingness or inability to comply with the requirements of the protocol.

3.2 Bone marrow harvesting, MSC culturing and injection

All selected patients underwent bone marrow aspiration under light general anaesthesia and an inoculum of crude bone marrow cells (150 mL) was obtained and two thirds of it was frozen. One third was cultured under GMP conditions at the human cell cultures clean room facility of Hadassah HMO. The MSCs were obtained from the bone marrow of each patient and prepared using a previously described protocol with slight modification [37].

One month later the participants were hospitalized and a lumbar puncture was performed under standard conditions and local anaesthesia at the L4-5 lumbar level; 3 mL of CSF were removed and the cultured purified MSCs (1 × 10^6/kg of body weight) resuspended in 3 mL of normal saline and injected into the CSF, using a 20-gauge needle and 3-way cannula.
Fig. 1. Flowchart of the trial. *: Patients were either lost to follow up and/or stopped treatments due to their inability to travel to our Center for further infusions (USA residences) or due to their unwillingness to undergo additional lumbar punctures.

3.3 Statistical analysis

The rate of progression was calculated based on the ALSFRS-R and FVC scores per month during the run-in period and at each time point of MSC-injection and follow up visit, for up to 2-years. Two-tailed Wilcoxon signed rank and Man-Whitney tests were used to compare the changes in the rates of progression in each patient during the run-in period and after each MSC-treatment and over the whole period of the study. The rationale for such comparison, derives from the fact that progression in ALSFRS-R was shown to be linear during the course of the disease, in large cohorts [69–73]. Additionally, a Kaplan-Meyer progression curve was calculated defining as treatment-failure each patient who progressed/deteriorated by more than 2 degrees in ALSFRS-R.

4. Results

4.1 Patients

Patients demography is shown in Table 1, which summarizes the baseline parameters of ALS, i.e., disease duration, baseline ALSFRS-R score, rate of progression before enrolment, and ALS prominent symptomatology (neurological involvement at the onset of ALS). Patients were allowed to continue other ALS treatments, i.e., Rizulole or Edravarone during the study. Summary of concomitant medications is shown on Table 1.

To evaluate the prognostic factors in the included patients, we calculated the rate of progression in ALSFRS-R from the onset of symptoms till the enrollment to the study or till the first MSC-treatment. As seen in Table 1, 18 out of the 20 included patients had a progression rate of more than 0.5 points in ALSFRS-R scale per month, indicating that this group consisted of patients with severe disease and bad prognosis [69–71, 73, 74].

4.2 Safety

We did not observe any serious side effects related to the treatment. 1 patient had aspiration pneumonia after the BMA, 1 suffered a fracture of the ankle, 1 had renal colic/uro lithiasis, all of them not related to the treatment with MSC. 3 patients suffered from headache and back pain after the stem cells injection, related to the lumbar puncture and 1 patient suffered from general weakness and fatigue for two weeks post MSC injection, possibly related to the treatment. All side effects were mild and resolved. One patient deceased 4 weeks after the first treatment, due to rapid deterioration of his disease and respiratory failure. The summary of all adverse events is shown on Table 2.

4.3 Clinical effects

Clinical analysis was performed in 19 out of 20 patients. Out of the 19 patients who underwent the first transplantation, 16 had a second injection, 12 a third injection and 10 patients had four cycles of MSC-transplantation. In total, 9 patients were lost to follow-up and stopped additional treatments between the four cycles of MSC-transplantation till the end of the trial, due to their difficulty to travel from abroad to our center or their unwillingness
| Patient number | Gender | Age (Years) | Disease duration (months) | ALSFRS-R at Baseline | Mean monthly progression rate from ALS onset to: | number of treatments | Concomitant medications | Main symptoms |
|----------------|--------|-------------|---------------------------|----------------------|-----------------------------------------------|-------------------|--------------------------|---------------|
| 001            | M      | 55.0        | 19.4                      | 34                   | Inclusion -0.617 Baseline -0.720              | 4                 | Lipitor                  | Gross Motor   |
| 002            | M      | 60.6        | 20.6                      | 26                   | -1.031                                          | 4                 | Riluzole, Edaravone      | Bulbar        |
| 003            | M      | 37.5        | 19.3                      | 34                   | -1.240                                          | 4                 | Riluzole                 | Gross Motor   |
| 004            | M      | 38.1        | 24.0                      | 32                   | -0.684                                          | 4                 | Riluzole, Edaravone      | Fine Motor    |
| 005            | M      | 50.9        | 18.1                      | 38                   | -0.552                                          | 1                 | Riluzole, Escitalopram   | Bulbar        |
| 006            | M      | 32.9        | 28.2                      | 24                   | -0.737                                          | 3                 | Riluzole, Nuedextra, Baclofen, Robinal | Bulbar        |
| 007            | M      | 42.1        | 28.4                      | 29                   | -0.459                                          | 4                 | -                        | Fine Motor    |
| 008            | M      | 45.6        | 24.3                      | 30                   | -0.776                                          | 2                 | Riluzole                 | Fine Motor    |
| 009            | F      | 49.7        | 20.6                      | 41                   | -0.317                                          | 4                 | Riluzole, Synthroid, Rizatriptan, Tazarotene | Gross Motor   |
| 010            | M      | 50.8        | 16.5                      | 23                   | -0.790                                          | 1                 | Riluzole                 | Bulbar        |
| 011            | M      | 44.5        | 18.1                      | 33                   | -1.425                                          | 2                 | Riluzole                 | Gross Motor   |
| 012            | F      | 51.6        | 20.7                      | 35                   | -1.117                                          | 4                 | Riluzole                 | Gross Motor   |
| 013            | F      | 59.9        | 20.0                      | 39                   | -0.495                                          | 4                 | Riluzole, Escitalopram, Clonazepam | Gross Motor   |
| 014            | M      | 66.0        | 8.1                       | 43                   | -1.064                                          | 3                 | Lipitor Aspirin          | Gross Motor   |
| 015            | M      | 57.8        | 15.0                      | 37                   | -0.543                                          | 2                 | Riluzole                 | Gross Motor   |
| 016            | M      | 55.2        | 9.2                       | 30                   | -2.299                                          | 2                 | -                        | Gross Motor (Familial) |
| 017            | M      | 50.4        | 13.3                      | 32                   | -1.729                                          | 1                 | Mesalazine, Loperamide, Lorazepam | Fine Motor    |
| 018            | M      | 48.1        | 34.9                      | 27                   | -0.186                                          | 8                 | Riluzole                 | Fine Motor    |
| 019            | F      | 47.1        | 26.0                      | 6                    | -1.606                                          | 0                 | Clonazepam, Mirtazapine, Esomeprazole, Glycopyrrolate, Citalopram, Quetiapine | Gross Motor   |
| 020            | M      | 55.5        | 27.4                      | 29                   | -0.600                                          | 4                 | Buspirone, Alprazolam    | Gross Motor   |
| Total          | 16-M   | Mean        | Mean                      | Mean                 | Mean                                            | Total             | 4: Bulbar                | 11: Gross Motor |
| Mean           | 4-F    | 49.97       | 20.61 m                   | 31.1                 | -0.937                                          | 61                | 5: Fine Motor            |               |
to undergo additional lumbar punctures and bone marrow aspiration, but they were included in the statistical analysis at each treatment point.

All the 20 recruited patients were followed before the transplantation for a mean period of 7.7 months (range: 3.5–18) and 19 out of 20 showed deterioration (reduction in the ALSFRS-R score during this period (mean monthly change: –1.036 ± 0.88).

The monthly rate of progression in ALSFRS-R was ameliorated (improved) by more than 25% (which arguably represents a clinically meaningful change) [75] in:

- 15/19 patients between the 1st and 2nd injection with mean improvement of 107.1%; three additional patients had an improvement of 4–15% and only one deteriorated.
- 11/12 patients between the 2nd and 3rd injection, with mean improvement of 91.4%.
- 8/10 patients between the 3rd and 4th injection.

Overall, during the whole period from the 1st to last transplantation:

- patients had a >25% improvement in the slope of progression of ALSFRS-R compared to the pre-treatment period with mean improvement of 47.4%.
- patients had an improvement of less than 25%.
- patients experienced a deterioration.

The overall beneficial effect was statistically significant (p = 0.0038, Mann and Whitney and Wilcoxon signed ranked test) (Fig. 2A,B and Tables 3A,3B).

As seen in Fig. 2A,B, there was a statistically significant reduction in the slope of progression after each one of all 4 cycles of transplantation, as compared to the deterioration during the pre-treatment period (−1.054 ± 0.86/month during the run-in period vs −0.051 ± 1.03 after the 1st transplantation, +0.019 ± 0.39 after the 2nd MSC-injection, −0.253 ± 0.26 after the 3rd injection and overall: −0.445 ± 0.81). 10 patients received 4 MSC injections, 2 received 3 injections, 4 had 2 injections and 3 one single injection (Table 3A). The progression rates during the whole period after the MSC-transplantations did not only differ from those in the run-in period but also seem to be significantly lower than those observed in large cohorts of ALS patients follow up [70, 71, 73, 74]. The response rate correlated with the time-intervals between the injections. Between the 3rd and the 4th MSC-injections, the reduction in the rate of deterioration in ALSFRS-R was still lower than the pre-treatment period, (−55.5%) but to a lesser degree than during the previous two periods of treatments (−107.1% and −91.4%) (Tables 3A,3B). We assume that the time intervals between the injections is important since the mean time between the 1st and 2nd injection and between the 2nd and the 3rd one, was 3.45 ± 0.6 and 3.14 ± 0.6 months, respectively, whereas between the 3rd and the 4th injection, 5.44 ± 1.7 months.

The Kaplan-Mayer survival curve that demonstrates the incidence of ALSFRS-R-progression of more than two degrees, is shown in Fig. 2C. A statistically significant difference in the curve, was observed comparing the post- and pre-treatment periods (p = 0.049, Log Rank, Martel-Cox).

Moreover, 7 out of 19 patients actually improved clinically (mean increase in ALSFRS-R: 2.86 ± 0.9; range of improvements: +2 to +4 degrees) after the first transplantation and 5 remained improved after the 2nd cycle (mean increase in ALSFRS-R: +2.60 ± 1.5, range of improvements: +1 to +4 degrees) and 3 remained with higher ALSFRS-R compared to baseline even after the third injection (mean increase in ALSFRS-R: 2.75 ± 0.9, mean time from baseline visit: 8.6 ± 1.7 months); 2 additional patients remained with the same ALSFRS-R score during the whole period.

The changes in respiratory function (FVC) are seen in Table 3B. Data are shown in 11 patients for which there were measurements at all time points up to the 3rd transplantation. There was a trend of beneficial effect (me-
Fig. 2. Clinical effects of repeated intrathecal MSC transplantations on the progression of ALS. (A) Follow up of ALSFRS-R before and after MSC treatments. *: $p < 0.05$, Mann and Whitney test. **: $p < 0.01$, Mann and Whitney test. (B) Monthly rates of ALSFRS-R changes before and after repeated intrathecal MSC treatments. *: Wilcoxon signed ranked test. (C) Kaplan-Meier survival curve based on the occurrence of progression in ALSFRS-R of at least 2 degrees (during the pre-treatment run-in period and the post treatment period following repeated intrathecal MSC injections).

5. Discussion and conclusions

The current single center open clinical trial, shows that repeated intrathecal injections of MSC in ALS patients was safe and well-tolerated, at least in the short/medium term. 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 on treatment with MSC in a variety of diseases, including ALS [33–44, 48, 63]. Although our study was primarily targeted to assess safety and not efficacy, our data provide indications of clinically meaningful beneficial effects induced by the repeated intrathecal injections of MSC. These are reflected by the slower rate of disease progression during the months following each transplantation vs the run-in pre-treatment period and the clinical improvements observed in several patients. On the basis of an individualized per patient analysis, the vast majority
### Table 3A. ALSFRS-R score progression (monthly change).

| Patient number | ALSFRS-R change | During run-in period between 1st & 2nd injection between 1st & 3rd injection between 1st & 4th injection | Overall |
|----------------|-----------------|-------------------------------------------------|---------|
| 1              | Monthly change  | −0.928                                          | 0       | −0.632 | −0.322 | −0.260 |
|                | Absolute change | 40→34                                          | 34→34   | 34→30  | 34→30  | 34→30  |
|                | Time (in months) (%) improvement in the rate of progression in ALSFRS-R | 6.47 | 3.07 (100%) | 6.33 (31.9%) | 12.43 (65.3%) | 15.37 (72.0%) |
| 2              | Monthly change  | −1.103                                          | 1.224   | 0.656  | −0.070 | −0.119 |
|                | Absolute change | 37→26                                          | 26→30   | 26→30  | 26→25  | 26→24  |
|                | Time (in months) (%) improvement in the rate of progression in ALSFRS-R | 9.97 | 3.27 (211.0%) | 6.10 (159.5%) | 14.23 (93.7%) | 16.77 (89.2%) |
| 3              | Monthly change  | −0.466                                          | 0.705   | 0.171  | −0.238 | −0.238 |
|                | Absolute change | 40→34                                          | 34→36   | 34→35  | 34→31  | 34→31  |
|                | Time (in months) (%) improvement in the rate of progression in ALSFRS-R | 12.87 | 2.83 (251.3%) | 5.83 (136.7%) | 12.60 (48.9%) | 12.60 (48.9%) |
| 4              | Monthly change  | −0.566                                          | 0.909   | 0      | −0.684 | −0.689 |
|                | Absolute change | 34→32                                          | 32→35   | 32→32  | 32→25  | 32→22  |
|                | Time (in months) (%) improvement in the rate of progression in ALSFRS-R | 5.35 | 3.30 (260.6%) | 7.23 (100%) | 10.23 (−20.8%) | 16.97 (−21.7%) |
| 5              | Monthly change  | −0.496                                          | 0       | 0      | 0      | 0      |
|                | Absolute change | 47→38                                          | 38→38   | 38→38  | 38→38  | 38→38  |
|                | Time (in months) (%) improvement in the rate of progression in ALSFRS-R | 14.13 | 2.33 (100%) | 2.33 (100%) | 2.33 (100%) | 0       |
| 6              | Monthly change  | −1.231                                          | 0       | 0      | 0      | 0      |
|                | Absolute change | 32→24                                          | 24→24   | 24→24  | 24→24  | 24→24  |
|                | Time (in months) (%) improvement in the rate of progression in ALSFRS-R | 6.5 | 3.27 (100%) | 6.53 (100%) | 8.60 (100%) |
| 7              | Monthly change  | −3.088                                          | 1.2     | 0.556  | 0.262  | 0.046  |
|                | Absolute change | 36→29                                          | 29→33   | 29→33  | 29→32  | 29→30  |
|                | Time (in months) (%) improvement in the rate of progression in ALSFRS-R | 2.27 | 3.33 (138.9%) | 7.20 (118.0%) | 11.47 (108.5%) | 21.73 (101.5%) |
| 8              | Monthly change  | −0.674                                          | −2.449  |        |        |        |
|                | Absolute change | 36→30                                          | 30→32   | 30→32  | 30→32  | 30→32  |
|                | Time (in months) (%) improvement in the rate of progression in ALSFRS-R | 8.9 | 3.27 (−263.4%) | 3.27 (−263.4%) | 3.27 (−263.4%) |
| 9              | Monthly change  | −0.411                                          | 0       | −0.158 | −0.335 | −0.260 |
|                | Absolute change | 43→41                                          | 41→41   | 41→40  | 41→37  | 41→20  |
|                | Time (in months) (%) improvement in the rate of progression in ALSFRS-R | 4.87 | 3.07 (100%) | 6.33 (61.6%) | 11.93 (18.5%) | 17.67 (36.7%) |
| 10             | Monthly change  | −3.097                                          | −1.622  |        |        |        |
|                | Absolute change | 39→23                                          | 23→9    |        |        |        |
|                | Time (in months) (%) improvement in the rate of progression in ALSFRS-R | 5.17 | 8.63 (47.6%) | 8.63 (47.6%) |
| 11             | Monthly change  | −0.309                                          | −0.214  |        |        |        |
|                | Absolute change | 36→33                                          | 33→32   | 33→32  | 33→32  | 33→32  |
|                | Time (in months) (%) improvement in the rate of progression in ALSFRS-R | 9.7 | 4.67 (30.7%) | 7.03 (54.0%) |
| 12             | Monthly change  | −0.368                                          | 0.677   | 0.347  | −0.122 | −0.122 |
|                | Absolute change | 40→35                                          | 35→38   | 35→38  | 35→33  | 35→33  |
|                | Time (in months) (%) improvement in the rate of progression in ALSFRS-R | 13.6 | 4.43 (284.0%) | 8.63 (194.3%) | 16.33 (66.8%) | 16.33 (66.8%) |
Table 3A. Continued.

| Patient number | ALSFRS-R change | During run-in period | between 1st & 2nd injection | between 1st & 3rd injection | between 1st & 4th injection | Overall |
|----------------|-----------------|----------------------|----------------------------|----------------------------|----------------------------|---------|
|                | Monthly change  | –0.339               | –0.288                     | –0.148                     | –0.191                     | –0.336  |
| 13             | Absolute change | 41→39                | 39→38                      | 39→38                      | 39→37                      | 39→35   |
|                | Time (in months) (% improvement in the rate of progression in ALSFRS-R) * | 5.9                     | 3.47 (15.0%)               | 6.73 (56.3%)               | 10.47 (43.7%)              | 11.90 (0.9%) |
| 14             | Monthly change  | –0.227               | –0.217                     | –0.366                     | –0.366                     | –0.366  |
|                | Absolute change | 44→43                | 43→42                      | 43→40                      | 43→40                      | 43→40   |
|                | Time (in months) (% improvement in the rate of progression in ALSFRS-R) * | 4.4                     | 4.60 (4.4%)                | 8.20 (–61.2%)              | 8.20 (–61.2%)              | 8.20 (–61.2%) |
| 15             | Monthly change  | –0.917               | –0.337                     | –                      | –                      | –0.837  |
|                | Absolute change | 44→37                | 37→36                      | 37→30                      | 37→30                      | 37→30   |
|                | Time (in months) (% improvement in the rate of progression in ALSFRS-R) * | 7.63                    | 2.97 (63.2%)               | –                      | –                      | 8.37 (8.7%) |
| 16             | Monthly change  | –1.912               | –1.667                     | –                      | –                      | –1.667  |
|                | Absolute change | 46→30                | 30→23                      | 30→23                      | 30→23                      | 30→23   |
|                | Time (in months) (% improvement in the rate of progression in ALSFRS-R) * | 8.37                    | 4.20 (12.8%)               | 4.20 (12.8%)               | 4.20 (12.8%)               | 4.20 (12.8%) |
| 17             | Monthly change  | –0.517               | +1.428*                    | –                      | –                      | 1.428   |
|                | Absolute change | 35→32                | 32→34                      | 32→34                      | 32→34                      | 32→34   |
|                | Time (in months) (% improvement in the rate of progression in ALSFRS-R) * | 5.8                     | 1.40 (376.2%)              | 1.40 (376.2%)              | 1.40 (376.2%)              | 1.40 (376.2%) |
| 18             | Monthly change  | –2.008               | –0.706                     | –0.415                    | –0.238                     | –0.221  |
|                | Absolute change | 43→27                | 27→25                      | 27→24                      | 27→24                      | 27→24   |
|                | Time (in months) (% improvement in the rate of progression in ALSFRS-R) * | 7.97                    | 2.83 (64.8%)               | 7.23 (79.3%)               | 7.23 (79.3%)               | 12.60 (88.1%) |
| 19             | Monthly change  | –1.392               | Withdrew                   | –                      | –                      | –       |
|                | Absolute change | 15→6                 |                            | 15→6                      |                            |         |
|                | Time (in months) (% improvement in the rate of progression in ALSFRS-R) * | 6.47                    |                            |                            |                            |         |
| 20             | Monthly change  | –1.029               | 0.30                       | 0.214                     | –0.595                     | –0.595  |
|                | Absolute change | 35→29                | 29→30                      | 29→30                      | 29→30                      | 29→30   |
|                | Time (in months) (% improvement in the rate of progression in ALSFRS-R) * | 5.83                    | 2.57 (137.9%)              | 4.67 (120.8%)              | 8.40 (42.2%)               | 8.40 (42.2%) |
|                | Monthly change  | –1.054 ± 0.86        | –0.051 ± 1.03              | +0.019 ± 0.39             | –0.253 ± 0.26              | –0.445 ± 0.81 |
|                | Average | Time (in months) (% improvement in the rate of progression in ALSFRS-R) * | 7.82 m | 3.55 m (107.1%) | 6.75 m (91.4%) | 12.07 m (55.5%) | 10.70 m (47.4%) |
|                | Confidence Interval (α = 5%; t-distribution) | (–1.46)–(–0.65) | (–0.51)–(+0.41) | (–0.16)–(+0.20) | (–0.38)–(–0.13) | (–0.62)–(–0.07) |

* % improvement in the rate of progression in ALSFRS-R during each post-treatment period, vs the run-in period.
Table 3B. Changes in FVC score.

| Patient | Pre-treatment run-in period: | Between 1st and 2nd injection: | Between 2nd and 3rd injection: | Pre-treatment run-in period: | Between 1st and 2nd injection: | Between 1st and 3rd injection: |
|---------|-----------------------------|-------------------------------|-------------------------------|-----------------------------|-------------------------------|-------------------------------|
|         | Delta FVC | Delta FVC | Delta FVC | Monthly FVC change | Monthly FVC change | Monthly FVC change |
| 001     | –12       | 0         | 0         | –1.85            | 0.00             | 0.00             |
| 002     | 0         | 1         | –1        | –1.40            | 0.30             | 0.00             |
| 003     | –11       | 1         | –1        | –0.70            | 0.35             | 0.00             |
| 004     | –8        | –10       | –5        | –2.26            | –3.03            | –2.07            |
| 006     | –15       | –5        | 3         | –2.31            | –1.53            | 0.45             |
| 007     | –3        | –3        | –5        | –1.32            | –0.90            | –1.11            |
| 009     | 0         | 0         | 0         | –0.82            | 0.32             | 0.00             |
| 012     | –9        | –1        | –3        | –0.15            | –0.22            | –0.46            |
| 013     | –3        | –6        | –3        | –0.50            | 0.86             | 0.00             |
| 014     | –6        | –6        | –4        | –0.45            | –2.60            | –1.95            |
| 018     | –24       | –8        | –23       | –3.01            | –2.82            | –4.28            |
| Mean ± SD | –8.27 ± 7.16 | –3.36 ± 3.85 | –3.82 ± 6.81 | –1.34 ± 0.92 | –0.84 ± 1.43 | –0.86 ± 1.41 |
| Median  | (8)       | (3)       | (3)       | (3)              | (3)              | (0)              |
| Confidence Interval (α = 5%; t-distribution) | (–13.08)–(–3.46) | (–5.95)–(–0.77) | (–8.39)–(–0.76) | (–1.96)–(–0.73) | (–1.80)–(0.11) | (–1.81)–(–0.09) |
| p value | 0.09      | 0.08      | NS        | NS               | NS               | NS               |

(65%) of the MSC-transplanted subjects were defined as responders, having at least 25% slower progression rate in ALSFRS-R after MSC-treatments, compared with the pre-treatment run-in period. Overall, during the whole period from the 1st to last transplantation 13 patients showed a >25% improvement in the slope of progression of ALSFRS-R (mean improvement of 47.4%); three had an improvement of less than 25% and only 3 a deterioration.

The rationale behind the approach of stem cell therapies in neurodegenerative diseases such as ALS, lies on the fact that CNS loses its capacity for efficient regeneration over time. This is especially pronounced in chronic neurodegenerative diseases, possibly due to an insufficiency of growth factors or defective mobilization of the intrinsic CNS stem cells/progenitors [76–79]. Based on their well described properties [76, 79–82], stem cells seem to represent a “logical” treatment approach to achieve those unmet needs and induce neuroprotection or neurotrophism. Moreover, stem cells are strong immunomodulators and may potentially downregulate inflammatory elements upon their migration to the CNS [83–85]. Inflammation was advocated to play a role in the progression of neurodegeneration even in diseases which were previously considered as “purely” degenerative, like ALS [86–88]. Several studies have shown that embryonic, neuronal, and other adult stem cells can induce beneficial clinicopathological effects in animal models of neurological diseases [7, 8, 21, 22, 89–92].

During the last decade, MSC treatments have been tested in various neurological diseases in small or pilot open-label trials [33–44], with promising indications.

The putative mechanism of action of MSC in neurological diseases is controversial. Some investigators claim that the most prominent effects are mediated through peripheral immunomodulation [4–6, 8, 78, 93, 94]. We have long advocated that neuroprotective and neurotrophic mechanisms play the most crucial role, as supported by our findings in animal models [7] and our pilot trials of MS and ALS [37, 41]. We speculate that IT injection, which brings a higher proportion of the injected cells into close proximity with damaged areas of the CNS, may induce more robust effects than intravenous injection as shown in our recent controlled trial in MS [64]. However, we cannot rule out possible immunomodulatory effects, that may also play a role in halting the progression of ALS. In this trial we haven’t performed an immunological analysis because we used only intrathecal administration and therefore peripheral effects are less likely, but based on our previous findings [37], such MSC-induced immunomodulatory mechanisms (probably mostly local in the CNS) may indeed contribute to the overall effect of MSC-treatment in ALS.

The main strength of our study is that this is a pivotal trial to evaluate the safety and clinical effect of repeated (up to four) injections of autologous MSC (every 3–6 months) and a follow-up period that exceeds that of previous studies. A controlled trial from a Korean group [48, 63] used two injections, very close one to another (26 days apart). In preliminary results from a recently completed phase III controlled study using 3 bimonthly injections of MSC-NTF (Nurown cells, Brainstorm®) the investigators reported that the study did not meet its main goal. While 34.7% of patients who received the stem cell therapy showed a numerical improvement according to the ALSFRS-R scale, that change did not differ significantly from the 27.7% in the placebo group, which also achieved the study goal. A subgroup of patients with baseline ALSFRS-R score of 35 or higher, showed improvement
that was “clinically meaningful” compared to those given a placebo. The analysis of cerebrospinal fluid confirmed a statistically significant increase in the nerve growth factors, as well as a reduction in biological indicators of neurodegeneration and inflammation, only in the stem cell-treated patients and not in the placebo group.

An additional strength of our study is related to the inclusion of patients with bad prognosis, as evidenced by the high rate of progression from onset of symptoms to inclusion to the study. The obvious limitations of our trial are the small number of patients and its open-label design. Being an open trial without a controlled group, an additional possible limitation of our findings could be theoretically related to a “regression to the mean” phenomenon. However, such regression, although may have affected the clinical changes at some degree, especially after the first transplantation and the first months of the study, cannot—to our view—explain the benefits observed during the subsequent cycles of treatment and the actual clinical improvements of several patients.

In summary, our results provide signals of, at least short/intermediate-term, clinical efficacy and possible indications of neuroprotection, induced by the repeated administrations of autologous MSC in patients with ALS. These data may contribute to the design of future trials with cell therapies. Larger studies are warranted to confirm our observations and further evaluate the therapeutic potential of cellular therapy in neurodegenerative diseases such as ALS.

6. Author contributions

PP—design and performance of the study, participation in writing of the manuscript; IK—design and performance of the study, participation in writing of the manuscript, analysis of the data. AG—design and performance of the study, participation in writing of the manuscript; IK—design and performance of the study, participation in writing of the manuscript, analysis of the data. NEY—design and performance of the study, participation in writing of the manuscript, analysis of the data.

7. Ethics approval and consent to participate

The study was approved by our Hospital Ethics committee (Approval number: 0208-16) and all participants signed an informed consent for the trial. ClinicalTrials.gov Identifier: NCT04821479.

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10. Conflict of interest

The authors declare no conflict of interest.

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