Safety and Efficacy of First-in-Man Intrathecal Transplantation of Human Astrocytes (AstroRx) in ALS Patients: Phase I/IIa Clinical Trial Results

Marc Gotkine
Hadassah Medical Organization: Hadassah University Medical Center

Yoseph Caraco
Hadassah Medical Organization: Hadassah University Medical Center

Yossef Lemer
Hadassah Medical Organization: Hadassah University Medical Center

Simcha Blotnick
Hadassah Medical Organization: Hadassah University Medical Center

Maor Wanounou
Hadassah University Hospital: Hadassah University Medical Center

Shalom Guy Guy Slutsky
Kadimastem Ltd

Judith Chebath
Kadimastem Ltd

Graciela Kuperstein
Kadimastem Ltd

eleena estrin
Kadimastem Ltd

Tamir Ben-Hur
Hadassah University Hospital: Hadassah University Medical Center

Arik Hasson
Kadimastem Ltd

Kfir Molakandov
Kadimastem Ltd.

Tehila Sonnenfeld
Kadimastem Ltd.

Yafit Stark
Kadimastem Ltd.

Ariel Revel
Kadimastem Ltd.

Michel Revel
Weizmann Institute of Science

Michal Izrael (✉️ M.izrael@kadimastem.com)
Abstract

Background: AstroRx is an allogeneic cell-based product, composed of healthy and functional human astrocytes derived from embryonic stem cells. We previously showed that AstroRx protects neurons in ALS animal models by multiple mechanisms, including clearance of toxic compounds (e.g. glutamate), reduction of oxidative stress, immunomodulation, and secretion of various neuroprotective factors. We hypothesized that transplantation of AstroRx can compensate for the malfunction of astrocytes in ALS patients in a clinical setting.

Methods: We conducted a phase I/IIa, open-label, dose-escalating clinical trial to evaluate the safety, tolerability, and therapeutic effects of AstroRx transplantation in patients with ALS. Five patients were injected intrathecally with a single dose of 100x10^6 AstroRx cells and 5 patients with 250x10^6 cells (low and high dose, respectively). Safety and efficacy assessments were recorded during a period of 3-months pre-treatment (run-in) and 12-months post-treatment (follow-up).

Results: A single administration of AstroRx at either low or high doses was safe and well tolerated. No adverse events (AEs) related to AstroRx cells were reported. Transient AEs related to the Intrathecal (IT) procedure were all mild to moderate and resolved. The study demonstrated a clinically meaningful effect that was maintained over the first 3 months after treatment, as measured by the pre-post slope change in ALSFRS-R. In the 100x10^6 AstroRx arm, the ALSFRS-R rate of deterioration was attenuated from -0.88/month pre-treatment to -0.30/month in the first 3 months post-treatment (p=0.039). In the 250x10^6 AstroRx arm, the ALSFRS-R slope from -1.43/mo to -0.78/mo (p=0.0023). The effect was even more profound in a rapid progressor subgroup of 5 patients. No significant difference in the rate of ALSFRS-R deterioration was observed beyond 3 months after treatment. No significant change was found in hand-held dynamometry (HHD), grip strength (JAMAR), ALSAQ-40, or serum biomarkers.

Conclusions: Overall, these findings suggest that a single IT administration of AstroRx to ALS patients at a dose of 100x10^6 or 250x10^6 cells is safe. A signal of beneficial clinical effect was observed for the first 3 months post cell injection. These results support further investigation of repeated IT administrations of AstroRx.

Trial Registration: NCT03482050

Background

Amyotrophic lateral sclerosis (ALS) is characterized by the loss of both upper and lower motor neurons (MNs). The symptoms include progressive paralysis of MN target muscles. The disease is incurable, and fatal within 3–5 years of first symptoms, usually due to respiratory failure when the diaphragm is affected\(^1\). The three FDA-approved drugs for the treatment of ALS, riluzole, edaravone, and the recently approved drug relivrio (a combination of sodium phenylbutyrate/taurursodiol) have a modest effect on survival and disease progression\(^2,3,4,5,6\), thus there is an urgent unmet need for therapies that can further delay the pathogenic process.
The pathological mechanisms for ALS are still not well understood. The proposed mechanisms include inflammation, oxidative stress, glutamate cytotoxicity, and protein aggregation. Although Motor Neurons (MNs) are the main affected cells in the disease, a growing body of evidence suggests the involvement of astrocytes in the pathogenesis of ALS in a non-cell-autonomous pathway. In healthy conditions, astrocytes support neurons in various ways. Astrocytes regulate the concentration of neurotransmitters and ions, supply a variety of metabolites and energy, regulate osmolarity, modulate synaptic activity, secrete neurotrophic and neuroprotective factors, promote neurogenesis and remyelination, and play a role in immunomodulation. The contribution of astrocytes to the pathology of ALS is probably a combination of loss of homeostatic functions and/or gain of toxic functions. Recent studies provide evidence for the beneficial role that astrocytes play in protecting MNs in ALS by reducing TDP-43 aggregates and secretion of neuroprotective factors. Interestingly, correction of a pathogenic germline mutation in astrocytes alone, slowed down MN degeneration. Comprehensive preclinical studies demonstrated that transplantation of glial-precursor-cells that were generated from iPSCs, or embryonic-stem-cells (ESC), had the potential to delay disease onset and ameliorate clinical symptoms in rodent models of ALS disease and shown to be safe. Thus, transplantation of healthy astrocytes into the CNS of ALS patients could potentially compensate for malfunctioning endogenous astrocytes and attenuate the progression of the disease.

Pluripotent human embryonic stem cells are an excellent source for regenerative therapies as they can be produced in high quantities and can differentiate into most cell types of the body, including astrocytes. Human astrocytes derived from clinical-grade embryonic stem cells (AstroRx) demonstrated activities of functional healthy astrocytes, including glutamate uptake, secretion of various neurotrophic factors (e.g. TIMP-1, TIMP-2, and Midkine), promotion of axon outgrowth, immunomodulation and protection of MNs from oxidative stress. Intrathecal injections of AstroRx into transgenic hSOD1G93A mice and rats significantly delayed disease onset and improved motor performance, as compared to control animals. A 9-month safety study in immunodeficient mice demonstrated the safety of AstroRx treatment, as well as the biodistribution and survival of the cells upon intrathecal administration.

Here we report on the results of a phase I/IIa, open-label, dose-escalating clinical study to evaluate the safety, tolerability, and therapeutic effects of AstroRx cells transplantation in patients with amyotrophic lateral sclerosis (ALS) (ClinicalTrials.gov Identifier: NCT03482050).

**Methods**

**Standard protocol approvals, registrations, and patient consent**

The study protocols were approved by the Israeli Ministry of Health (IMO), and the institutional review board of Hadassah Medical Center in Jerusalem, Israel. All patients signed informed consent documents before screening.

**Study Objectives**
The study aimed to evaluate the safety, tolerability, and therapeutic effects (preliminary efficacy) of a single intrathecal injection of low and high doses of AstroRx, as a treatment for patients with ALS.

**Patients' Selection Criteria**

Eligible participants were aged 18–70 years with a diagnosis of probable or definite ALS by revised El Escorial Criteria, within two years of diagnosis. The ALSFRS-R score was $\geq 30$, and slow vital capacity (SVC) $\geq 70\%$ of the predicted normal value for height, age, and sex. Participants were either not receiving riluzole and/or edaravone or were on a stable dose for $\geq 30$ days. Potential patients were to be excluded for the following reasons: past infection or a positive test for HBV, HCV, or HIV, need for respiratory support, renal failure, impaired hepatic function, Body Mass Index (BMI) of $<18.5$ or $>30$, significant cardiac disease, diabetes, autoimmune diseases, chronic severe infection, malignant disease or any other disease or condition that may risk the patient or interfere with the ability to interpret the study results, patient has been treated previously with any stem cell therapy. A full list of inclusion and exclusion criteria is shown in Supplementary 1.

**Study Outline**

A diagram of the study design is shown in Fig. 1a. The study was conducted under 2 sequential clinical protocols, the interventional protocol Astro-001 and its extension, non-interventional protocol, Astro-002.

Study Astro-001: following enrollment, the patients were monitored monthly during a run-in period of about 3 months to determine the progression rate of their ALS disease. Following the run-in period, patients were administered with $100 \times 10^6$ AstroRx cells (Group A, $n=5$ patients) and $250 \times 10^6$ cells (Group B, $n=5$ patients) by a standard LP procedure. The immunosuppressive drug, Mycophenolate Mofetil (MMF) at 1gr b.i.d. for 1 month was given 2 days before transplantation and continued for an additional one month (total of 32 days). Patients underwent weekly complete blood count (CBC) during the month of MMF treatment, and twice monthly following MMF cessation, to check for leukopenia.

The initial study design consisted of two additional arms of repeated doses of $100 \times 10^6$ cells and $250 \times 10^6$ separated by an interval of 60 days. However, due to the COVID-19 pandemic restrictions and the perception of the potential risks it posed to people with ALS, we adopted the recommendation of the independent Data Safety Monitoring Board (DSMB) and did not treat these 2 cohorts. After AstroRx cell transplantation, the patients were monitored monthly during a follow-up of 6 months. Upon completion of Study Astro-001 (at a 6-month follow-up), participants were offered enrolment in the extension study Astro-002.

Under the study Astro-002, each patient was followed up monthly for an additional 6 months by either on-site visits or phone calls. The outcome measures were similar to study Astro-001. Safety data were monitored by the study investigators, medical monitor, and the data and safety monitoring board (DSMB), which was independent of the study and sponsor.

**AstroRx Cell Manufacturing**
The Clinical-grade AstroRx cell product was manufactured under cGMP conditions in Kadimastem GMP facility using standard operating procedures. AstroRx cells were formulated in PlasmaLyte to reach a volume of 5ml with 100x10^6 or 250x10^6 AstroRx cells. The formulated drug product was uploaded into a 10 ml syringe and transported from the manufacturing facility to the clinical site in a validated shipping system at a controlled temperature of 2°C–8°C and administered fresh to the patient. Each shipment's temperature was recorded by a temperature logger. Safety Quality Control tests were performed prior to the release of each AstroRx product, including sterility, mycoplasma, endotoxin, and Gram or HBL test. The viability and cell concentration were determined using the automated cell counter Nucleocounter (NC-200™ Chemometec®). The identity of AstroRx cell product was assessed by flow cytometry using the following antibodies: anti-GLAST (Miltenibiotec, 1:100), anti- CD44 (BD Pharmingen, 1:50), and anti-GFAP (Miltenibiotec, 1:50). Antibodies against SSEA-4 and EPCAM (both from Biolegend) were used for the detection of any pluripotent marker impurities. The Flow cytometer FACS Canto II (BD) operated with FACSDIVA software (BD) was used for the analysis. To assess AstroRx potency in-vitro, the ability of AstroRx cells to secrete Midkine as well as TIMP-1 was determined by ELISA using Human TIMP-1 Quantikine ELISA Kit (R&D systems) and Human Midkine ELISA Kit (Abcam). The optical density was read using the iMark Microplate reader (Bio-Rad Laboratories). A certificate of analysis was generated and approved by the quality assurance department to ensure that each released product met the release criteria.

**Measurement Outcomes**

The primary objective of this study was to assess the safety and tolerability of AstroRx in patients with ALS. Safety laboratory assessments and adverse events (AEs) were collected at each visit, in addition, CNS imaging by MRI and CT were performed around 1 month before treatment and 1 and 6 months after treatment.

The second objective was to evaluate the efficacy of AstroRx cell transplantation in ALS. For this aim, data of ALSFRS-R, slow vital capacity (SVC), hand-held dynamometry (HHD), and grip strength (using JAMAR plus) were collected at all pre-treatment and post-treatment on-site visits. ALSFRS-R score also performed during home and phone visits. All tests were performed by trained evaluators who were certified by the Outcomes and Monitoring Center for the Northeast ALS Consortium. In addition, levels of the serum biomarker creatinine, creatine kinase, and Nfl were assessed in selected visits before and after treatment (supplementary 1).

**Serum Neurofilament Analysis**

Serum samples for the analysis of Nfl as a biomarker for ALS progression were collected from patient in groups A and B. In study Astro-001-IL, samples were collected on Visit 2 (day − 60), Visit 3 (day − 30), Visit 4a (day − 1), Visit 4d (day + 1), Visit 5 (day + 30), Visit 7 (day + 90) and Visit EOS (day + 180). In the extension study Astro-002-IL, samples were collected at the Screening Visit (day + 180; EoS Visit in protocol Astro-001-IL), Visit 9M (day + 270), and EOS Visit (day + 365). The measurement of the concentration of the biomarker in serum was performed using the validated SIOMA (Single Molecule Array) method by Quanterix Ltd. (US).

**Statistical analysis**
The primary trial outcome was safety, assessed with respect to the incidence of treatment-emergent AEs (TEAEs) and serious AEs (SAEs), laboratory abnormalities, vital signs, ECGs, and physical examinations. AEs were coded using MedDRA. Secondary endpoints were comparisons of (1) the change in slopes; post-treatment slope over 12 months, as compared to the pre-treatment slope (“Run-in’) in ALSFRS-R and SVC. The efficacy analyses used a repeated mixed model with fit least squares (LS) means as well as responder analyses comparing AstroRx pretreatment and post-treatment period.

Results

Twenty-four patients were screened in this clinical study. Six patients were screen failures, and an additional 2 patients were screened but not enrolled, due to study discontinuation following the COVID-19 pandemic outbreak. Five patients enrolled in Group A (a single administration of 100x10^6 AstroRx cells). Four patients completed the 6-month follow-up under protocol Astro-001-IL, and 3 of them continued to the extension study Astro-002-IL and completed the entire 12-month follow-up after treatment. Eight patients enrolled in Group B (a single administration of 250x10^6 AstroRx cells) and 5 patients were treated (Figure 1b). All 5 patients completed the 6-month follow-up and continued to the extension study, and 3 of them completed the entire 12-month follow-up (Figure 1b).

Prior to its injection, each AstroRx product was tested for the number of cells, viability, sterility profile, astrocytic cell identity, impurities, and potency to ensure meeting the release criteria defined for clinical batches (Table 1). Three patients enrolled in group C (2 administrations of 250x10^6 AstroRx cells). However, due to the COVID-19 outbreak, it was decided to discontinue the study for Group C, and no patient was treated in this group.

The baseline characteristics of the patients are presented in Table 2. Nine of the 10 treated patients of Group A and Group B were male, and all patients were white. All patients were stable on riluzole and none was treated with edaravone. The average age of the patients was 63±4.9 in Group A and 61±6.2 in Group B. All 5 patients in Group A had a diagnosis of probable ALS by El Escorial Criteria. In Group B, 3 patients had a diagnosis of probable ALS and 2 patients had a diagnosis of definite ALS. Nine of the 10 patients reported limb-onset of the disease and 1 patient (Group B) reported a bulbar onset. The time from first symptoms was 18.8±5.7 and 17.1±5.3 months for Group A and Group B, respectively. The time from diagnosis was 14.5±4.6 and 10.6±2.0 months for Group A and Group B, respectively.

Safety

Nine out of 10 (90%) of treated patients completed the 6-month follow-up, and 6 patients (60%) completed the 12-month follow-up. One patient in Group A and 2 patients in Group B died during the study, between 9 to 10 months post-treatment, due to respiratory failure that was associated with the natural progression of ALS. Table 3 summarizes the treatment-emergent adverse events (TEAE) reported in the study. All patients reported a total of 87 treatment-emergent adverse events (TEAE). None of TEAE was deemed to be associated with AstroRx treatment. Sixty-three TEAEs were mild, 19 were moderate, and 4 were severe. None of the TEAEs led to early discontinuation. Six patients developed a total of 9 serious TEAE after the treatment, 2 patients in Group A and 4 patients in Group B (Supplementary 2; eTable 1). The most common TEAEs that were reported
by at least 20% of the patients from Group A and Group B are shown in eTable 2. The Most frequent TEAE was post lumbar puncture (IT) headache, associated with IT injection procedure of the cells, and reported by 50% of the patients. Additional procedure-related TEAEs included pain in the injection site (30%), arthralgia, back pain, muscle contraction, and pain in the leg, each reported by 10% of the patients (eTable 3). All procedure-related AEs were graded as mild to moderate, and all were resolved. One event of moderate post-LP headache was resolved following a blood patch procedure that required hospitalization and was classified as an SAE. There was no apparent difference in the frequency or the nature of the procedure-related AEs between treatment groups. Three patients reported 4 AEs were related to mycophenolate mofetil, including headache, nausea, anemia, and hyperhidrosis (eTable 4). All the immunosuppression-related AEs were graded as mild to moderate, and all were resolved. No clinically significant changes were observed throughout the study in laboratory assessments, as well as in vital signs, physical examinations, or ECG results. MRI scans of the brain and spinal cord performed 6 months after AstroRx cell injection showed no tumor formation in the CNS. Results were similar also after 12 months of follow-up, however, the MRI data at 12 months were limited due inability of patients to perform MRI due to their medical condition, and restrictions imposed by the COVID-19 pandemic.

**Efficacy**

**ALSFRS-R**

The main outcome efficacy measure in the study was ALSFRS-R. At baseline visit (1 day before treatment) the mean ALSFRS-R score was 35.6±3.7, 34.2±7.0, 34.9±5.3, and 33.4±6.4 for Group A, Group B, combined Group A+B, and Rapid Progressors, respectively. The mean decline in the ALSFRS-R slope for patients in Group A was -0.88/month during the run-in (3-4 months prior to treatment). In the first 3 months after AstroRx cell injection, the mean decline of the ALSFRS-R slope was attenuated to -0.3/month (p=0.039), reflecting an attenuation of 66% in ALSFRS-R deterioration (Figure 2). At 6 and 12 months after treatment, the ALSFRS-R deterioration rate was -0.76/month and -0.82/month, respectively – similar to that observed during run-in (Figure 2). The mean deterioration of ALSFRS-R slope in Group B (-1.43/month) during the run-in was greater than Group A (-0.88/month). Similar to Group A, the ALSFRS-R deterioration rate during the first 3 months after treatment decreased to -0.78/month (p=0.002), representing an attenuation of 45% in ALSFRS-R decline. As observed in Group A, the attenuation of ALSFRS-R decline over the first 3 months post-treatment was not maintained at 6 and 12 months post-treatment (-1.59/month and -1.39/month, respectively) (Figure 2). Combining the data of both groups demonstrated an attenuation of 53% in ALSFRS-R over the first 3 months post AstroRx IT injection (p<0.001), which was not maintained at 6- and 12-month follow-up (Figure 2). The change in ALSFRS-R slope was also analyzed in a subpopulation of rapid progressors from both groups (n=5). Rapid progressors were defined as patients who deteriorated ≥1.1 ALSFRS-R points per month during the run-in 25,26. The mean improvement in ALSFRS-R slope between run-in and 3-month follow-up in these patients was 58% (-1.58/month vs. -0.65/month, p<0.001). Also in this subpopulation, after 3 months post single dosing the ALSFRS-R slope returned to a similar rate that was recorded prior to treatment (Figure 2). An improvement ≥25% in ALSFRS-R slope is considered clinically meaningful 27. The individual ALSFRS-R slopes (Supplementary; eFigure 1) demonstrated an improvement of at least 25% in ALSFRS-R slope between run-in and 3-month follow-up in 80% of the patients (4 patients in each group, data not shown).
Hand-held dynamometry (HHD)

A comparison of the HHD megascore slope between run-in and 3-month follow-up showed a trend of improvement in both Group A and Group B, which was not statistically significant (eTable 5). In combined Group A+B, the rate of HHD megascore decline in the run-in was -0.06±0.028 vs. -0.02±0.031 during the first 3-months post-treatment (p=0.24) and in Rapid Progressors -0.06±0.053 vs. +0.01±0.060. At 6- and 12-month follow-up, the HHD megascore returned to the similar decline rate that was recorded in the run-in.

Slow vital capacity

At the baseline visit, the mean percent of predicted SVC (%SVC) was 77.9±14.2%, 67.8±18.9%, 72.9±16.6%, and 66.6±17.1% for Group A, B, combined A+B, and Rapid Progressors, respectively. A comparison of %SVC rate deterioration between run-in and follow-up showed a continuation of %SVC deterioration in both Group A and Group B, and Rapid Progressors (eTable 6). The rate of decline in %SVC rate in combined Group A+B (n=10) was −1.08±1.04% in run-in vs. -3.20±1.10% during the first 3 months of follow-up (p=0.01), and -3.09±0.59 during the 12-month follow-up (p=0.01).

Serum neurofilament light chain (NfL)

Serum samples for the analysis of NfL as a biomarker for ALS disease progression were collected throughout the study, before and after treatment. NfL, which was extensively studied in ALS, is proposed as a potential biomarker for ALS diagnosis and prognosis. The proposed cut-off serum NfL level to differentially distinguish between ALS patients vs. non-neurodegenerative controls is 49 pg/mL. The serum NfL concentration in six of the patients was greater than 49 pg/ml throughout the study and levels tended to be higher in rapid progressors, as reported by others (data not shown). However, no clear tendency of change in the kinetics of serum NfL was observed (eFigure 2).

Discussion

Although the pathogenesis of MN death in ALS is not fully elucidated, malfunctioning astrocytes can contribute to the death of MNs and the progression of the disease. A cell therapy approach that includes the transplantation of healthy and functional astrocytes may compensate for the diseased endogenous astrocytes and attenuate the disease progression.

AstroRx cell therapy is composed of healthy astrocytes derived from human embryonic stem cells. Intrathecal injection of AstroRx allows the distribution of the cells throughout the neural axis, where it can affect both upper and lower MNs. Moreover, AstroRx is an allogenic “off-the-shelf” product that will potentially enable ALS patients to benefit from this treatment without the requirement of individual production procedures of autologous cells.

This first-in-human phase I/IIa clinical trial assessed the safety and preliminary efficacy of a single intrathecal injection AstroRx in two doses. Ten patients were enrolled into the study, 5 in each treatment dose. No AEs related to the product itself were reported. The most common AEs were related to the intrathecal
administration procedure or treatment with MMF. These AEs were transient, mild to moderate, and all resolved either spontaneously or with treatment.

In this first-in-human phase I/IIa clinical trial the safety and preliminary efficacy of a single intrathecal injection of AstroRx was tested in two doses.

Ten patients were enrolled into the study, 5 in each treatment dose. No AEs related to the product itself were reported. The most common AEs were related to the intrathecal administration procedure or treatment with MMF. These AEs were transient, mild to moderate, and all resolved either spontaneously or with treatment.

Reported SAEs were related to the expected progression of ALS. Three patients died due to the natural progression of ALS 9 to 10 months post-treatment.

A potential major safety concern in using embryonic stem cells as a source for cell therapy is their potential to form teratomas. Prior to its transplantation each AstroRx cell product was tested to meet the acceptance criteria for pluripotent markers. In the clinical trial MRI scans of the spinal cord and brain performed 6 months after cell injection did not reveal any tumor or teratoma formation. Overall, these safety data indicate that a single injection of AstroRx at both tested doses of $100 \times 10^6$ and $250 \times 10^6$, is safe and well-tolerated.

The patient population enrolled in this study was at a relatively early disease stage (about 18 months from first symptoms) and the ALSFRS-R at baseline was similar between groups. The percentage of male patients in the study was 90%, profoundly greater than the estimated ALS incidence ratio of 1.29:1 between males and females. Although the known mechanisms of action of AstroRx are assumed to influence similarly both genders, in future larger clinical studies, efforts will be made to include patients that better reflects the gender ratio in the ALS population. The disease progression recorded during the run-in was on average greater in patients of Group B. The progression of ALS was assessed by pre-post analysis of slope analysis, change from baseline, and responder analysis. The analyses were performed also on a subpopulation of rapid progressors (ALSFRS-R $\geq 1.1$ per month during run-in). A clinically meaningful signal of decline in disease progression, as assessed by the ALSFRS-R score, was observed for the first 3 months after treatment, as compared to the pre-treatment period. Although the deterioration in Group B patients was greater than that of Group A, the trend of effect was similar. A similar trend was also observed in the rapid progression population suggesting that AstroRx has the potential to be effective in a broader ALS patient population. The additional outcome measures of Muscle strength as measured by HHD showed a trend of slowdown in deterioration for the first 3 months post treatment as compared to run-in period but was not statistically significant. In contrast, respiratory function expressed as predicted SVC continued to deteriorate during the entire follow-up, including the first 3 months post AstroRx injection. No clear trend of change was observed in serum marker NfL between pre- and post-treatment periods. The interpretation of the efficacy results is limited by the small sample size and the difference in disease progression prior to treatment between study groups. Yet the trend of attenuation in disease progression for the first 3 months as reflected by ALSFRS-R was observed in 8 out of 10 patients from both groups. Notably, in larger studies supporting FDA market authorization, ederavone and the recently ALS approved drug and reliviro, showed a modest but statistically significant benefit in slowing down ALSFRS-R decline, although they did not demonstrate a significant improvement in other ALS outcome measures. The effect of AstroRx on ALSFRS-R, as well as the other
ALS outcome measures, should be further evaluated in a larger randomized parallel, placebo-controlled clinical trial.

The duration of effect of AstroRx may be related to the survival of the cells in the CNS. In preclinical study in immunodeficient mice, AstroRx cells were shown to survive in the CNS at the endpoint of the study, 9 months after their intrathecal injection\(^\text{19}\) (Izrael 2018). Since the CNS is not considered as a fully immune privileged site\(^\text{35, 36}\) transplantation of allogeneic cell product in the CNS in clinical trials are usually accompanied by immunosuppression to prevent any possible immune attack against the transplant\(^\text{37, 38}\). In our study, a prophylactic regimen of MMF was given for a duration of one month post dosing. The survival of the cells in the CNS of the patient was not investigated in this clinical study. It cannot be excluded that the reduction in the clinical signal of effect after 3 months is a result of loss of AstroRx cells. Nevertheless, we do not have an indication for a systemic immune response following cell transplantation as measured by blood immunoglobulins or change in -interferon release by peripheral blood mononuclear cells (PBMC) collected from the treated patient before and after treatment (data not shown). The survival of AstroRx cells post transplantation and the regimen of immunosuppression will further be explored in the next clinical study.

In conclusion, a single IT administration of AstroRx, an astrocyte cell-based therapy derived from embryonic stem cells, at a dose of 100x10\(^6\) or 250x10\(^6\) cells is suggested to be safe. A signal of beneficial clinical effect was observed over the first 3 months post single treatment. It remains to be investigated whether repeated IT administrations of AstroRx may prolong its beneficial effect in ALS.

**Abbreviations**

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AE</td>
<td>adverse event</td>
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<td>ALS</td>
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<td>ALSFRS-R</td>
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CK  creatine kinase
CNS  central nervous system
CNTF  ciliary neurotrophic factor
Cr  creatinine
CSF  cerebrospinal fluid
C-SSRS  Columbia-Suicide Severity Rating Scale
CT  computed tomography
CXCR  C-X-C chemokine receptor
dSMB  data safety monitoring board
eCRF  electronic case report form
EC  ethics committee
ELISA  enzyme-linked immunosorbent assay
FU  follow-up
GDNF  glial derived neurotrophic factor
GFAP  glial fibrillary acidic protein
GLAST  glutamate aspartate transporter
hESC  human embryonic stem cells
HHD  hand-held dynamometer
ITT  intention to treat
IT  intrathecal
LP  lumbar puncture
MedDRA  Medical Dictionary for Regulatory Activities
mITT  Modified Intent to Treat
MMF  mycophenolate mofetil
MMRM  Mixed-effect model for repeated measures
Declarations

Ethics approval and consent to participate

The study protocols and inform consent forms were approved by the Israeli Ministry of Health (IMOH), and the institutional review board of Hadassah Medical Center in Jerusalem, Israel. All patients sign informed consent after receiving oral and written information and could withdraw from the study at any time point. The studies were performed in compliance with the World Medical Association Declaration of Helsinki and ICH E6 for Good Clinical Practice.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no conflicts of interest.

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Authors' contributions
MG, YC, YL, MW: Clinical investigator, contribution to protocol design, conduct the study, and writing the manuscript. SB: Pharmacist of the study. TBH: Consultant for the design of the study and writing manuscript. SGS: Clinical Project Manager, writing of manuscript, and design and execution of the clinical protocol. MI: Head of R&D, Supervise all activities, writing of manuscript, and design of clinical protocol. AH: Design of clinical protocol and establishment of study infrastructures. YS: Global clinical development advisor. GK, EE: QA and QC of the AstroRx. TS: AstroRx GMP manufacturing. JC, KM: Quality control support. AR: Medical advisor for the study. MR: AstroRx invention and development, and design of the clinical protocol.

Acknowledgements

Not applicable

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request

References


### Tables

**Table 1: Cell information**

**Part 1: Cell number, Viability and Safety profile:**
Part 2: AstroRx cell characteristics

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<th>Cell Characteristics</th>
<th>Cohort (Av±SEM)</th>
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</tr>
<tr>
<td>CD44 (%)</td>
<td>99.3±0.2</td>
<td>99.8±0.0</td>
</tr>
<tr>
<td>GLAST (%)</td>
<td>88.8±7.6</td>
<td>69.9±1.3</td>
</tr>
<tr>
<td>GFAP (%)</td>
<td>97.5±0.9</td>
<td>92.2±1.3</td>
</tr>
<tr>
<td>Potency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIMP-1 (ng/10^6)</td>
<td>38.2±7.7</td>
<td>43.9±6.1</td>
</tr>
<tr>
<td>MIDKINE (ng/10^6)</td>
<td>14.3±1.3</td>
<td>19.9±2.0</td>
</tr>
</tbody>
</table>

Abbreviations: NC; No Contamination, EU; Endotoxin Unit, EPCAM; Epithelial Cell Adhesion Molecule, SSEA-4; Stage-Specific Embryonic Antigen-4, CD44; Cluster of Differentiation 44, GLAST; Glutamate Aspartate Transporter and GFAP; Glial fibrillary acidic protein.

**Table I: AstroRx cell information.** Part 1: Cell number, Viability and Safety profile of AstroRx cells used for Intrathecal injection for each ALS patient. Part2: an average of AstroRx cell characteristics used for each experimental cohort.

**Table 2: Baseline Demographics**
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>A (n=5)</th>
<th>B (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5 (100%)</td>
<td>4 (80%)</td>
</tr>
<tr>
<td>Female</td>
<td>0</td>
<td>1 (20%)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>5 (100%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>63 (4.9)</td>
<td>61 (6.2)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173.4 (6.0)</td>
<td>170.6 (11.7)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65.2 (15.4)</td>
<td>72.8 (8.1)</td>
</tr>
<tr>
<td>BMI (m&lt;sup&gt;2&lt;/sup&gt;/kg)</td>
<td>21.6 (4.3)</td>
<td>25.2 (4.3)</td>
</tr>
<tr>
<td>ALS Diagnostic Criteria (revised El-Escorial)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Definite</td>
<td>0.0</td>
<td>2 (40%)</td>
</tr>
<tr>
<td>Probable</td>
<td>5 (100%)</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>Time from First Symptom (months)</td>
<td>18.8 (5.7)</td>
<td>17.1 (5.3)</td>
</tr>
<tr>
<td>Time from Diagnosis (months)</td>
<td>14.5 (4.6)</td>
<td>10.6 (2.0)</td>
</tr>
<tr>
<td>Riluzole Use</td>
<td>5 (100%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>Initial Symptom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulbar Onset</td>
<td>0</td>
<td>1 (20%)</td>
</tr>
<tr>
<td>Limb Onset</td>
<td>5 (100%)</td>
<td>4 (80%)</td>
</tr>
<tr>
<td>ALSFRS-R</td>
<td>35.6 (3.7)</td>
<td>34.2 (6.98)</td>
</tr>
<tr>
<td>% Predictive SVC</td>
<td>77.9 (14.2)</td>
<td>67.8 (18.9)</td>
</tr>
<tr>
<td>Grip Strength (Kg.Force)</td>
<td>8.4 (13.0)</td>
<td>16.0 (15.8)</td>
</tr>
<tr>
<td>HHD Mega Score</td>
<td>-1.36 (0.42)</td>
<td>-0.52 (1.42)</td>
</tr>
</tbody>
</table>

Data are n (%) or mean (SD).

**Table 3: Summary of TEAEs**
<table>
<thead>
<tr>
<th>Category</th>
<th>A (N=5)</th>
<th></th>
<th>B (N=5)</th>
<th></th>
<th>A+B (N=10)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients n (%)</td>
<td>Events n</td>
<td>Patients n (%)</td>
<td>Events n</td>
<td>Patients n (%)</td>
<td>Events n</td>
</tr>
<tr>
<td>Any TEAE</td>
<td>5 (100)</td>
<td>54</td>
<td>5 (100)</td>
<td>32</td>
<td>10 (100)</td>
<td>86</td>
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<tr>
<td>Death</td>
<td>1 (20)</td>
<td>2 (40)</td>
<td>2</td>
<td></td>
<td>3 (30)</td>
<td></td>
</tr>
<tr>
<td>Any Serious TEAE</td>
<td>2 (40)</td>
<td>5</td>
<td>4 (40)</td>
<td>4</td>
<td>6 (60)</td>
<td>9</td>
</tr>
<tr>
<td>Any Severe TEAE</td>
<td>1 (20)</td>
<td>2</td>
<td>2 (40)</td>
<td>2</td>
<td>3 (10)</td>
<td>4</td>
</tr>
<tr>
<td>Any TEAE related to study drug</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Any TEAE related to IT Procedure</td>
<td>3 (60)</td>
<td>6</td>
<td>4 (80)</td>
<td>6</td>
<td>7 (70)</td>
<td>12</td>
</tr>
<tr>
<td>Any TEAE related to immunosuppression</td>
<td>1 (20)</td>
<td>2</td>
<td>2 (40)</td>
<td>2</td>
<td>3 (30)</td>
<td>4</td>
</tr>
<tr>
<td>TEAEs Severity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>5 (100)</td>
<td>41</td>
<td>5 (100)</td>
<td>22</td>
<td>10 (100)</td>
<td>63</td>
</tr>
<tr>
<td>Moderate</td>
<td>4 (80)</td>
<td>11</td>
<td>4 (80)</td>
<td>8</td>
<td>8 (80)</td>
<td>19</td>
</tr>
<tr>
<td>Severe</td>
<td>1 (20)</td>
<td>2</td>
<td>2 (40)</td>
<td>2</td>
<td>3 (10)</td>
<td>4</td>
</tr>
</tbody>
</table>

Figures
A. Scheme of study

Phase 1/2a study design (A) and study flowchart (B).

V=Visit, mo=Month, BCV=Blood Count Visit, EOS=End of Study

Phase 1/2a study design and study flowchart. A. Visit 0 (V0) - screening visit, visit 1 (V1) till visit 4 (V4) presents about 3 months run-in period (pre-treatment), AstroRx injection was performed on V4. V4 till visit V10 is the 6 months follow up time under ASTR0-001 study and additional 6 months follow up was performed.
under study ASTRO-002 on V10-V12 and by phone call. B. Study flow chart of patient allocation, treatment doses of ASTRO-001 and ASTRO-002. V=Visit, mo=Month, BCV=Blood Count Visit, EOS=End of Study.

Figure 2

ALSFRS-R slopes analysis in run-in, and 3-, 6- and 12-month follow up after AstroRx® treatment

ALSFRS-R slopes analysis in run-in, and 3-, 6- and 12-month follow up after AstroRx® treatment. The change in slopes between pre-treatment slope (“Run-in”) and post-treatment slope over 12 months was analyzed by using a repeated mixed model with fit least squares (LS) means. Analysis was performed on Cohort A, Cohort B, Cohort A&B as well as on rapid progressors (defined by ALSFRS-R ≤ 1.1/month during run-in). * = P value=0.039 (Run-in vs. 3-month FU, & = P value=0.002 (Run-in vs. 3-month FU) and # = P value <0.001 (Run-in vs. 3-month FU).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementary1JTM.docx
- Supplementary2eFiguresandeTables.docx