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## LETTERS TO THE EDITOR

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### RARE COMBINATION OF MYASTHENIA AND MOTOR NEURONOPATHY, RESPONSIVE TO MSC-NTF STEM CELL THERAPY

A 75-year-old man was referred to the Hadassah Medical Center with a 6-month history of progressive limb weakness, dysarthria, and cognitive deterioration. His past medical history included prostate hyperplasia, hypothyroidism, diabetes mellitus, cardiac arrhythmias controlled by pacemaker implantation (1997), and hypertension. Autoimmune myasthenia gravis (MG) had been diagnosed 2 years earlier, based on symptoms of fluctuating fatigue, dysarthric speech, eyelid ptosis, and seropositivity for muscle acetylcholine receptor (AChR) binding antibody. MRI did not reveal thymic enlargement, and malignancy markers (and whole-body computed tomography) were negative. Moderate improvement followed treatment, initially with intravenous immune globulin and later with low dose corticosteroids, pyridostigmine, and azathioprine. The patient was evaluated previously at the Mayo Clinic (Rochester, Minnesota) and had been diagnosed with amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The diagnosis was based on the cognitive exam and clinical features of progressive diffuse upper and lower motor neuron dysfunction with electromyographic findings (low motor amplitudes, no focal slowing or conduction blocks, no postexercise facilitation, presence of diffuse fibrillation and fasciculation potentials, and large complex motor unit potentials in all limbs) fulfilling the El-Escorial criteria for ALS.<sup>1</sup> MRI and positron emission tomography scans of the brain showed moderate atrophy predominantly affecting the frontal and temporal lobes. Spinal fluid was acellular with slightly elevated total protein (50 mg%). Autoimmune serology revealed antibodies specific for muscle (AChR binding 9.47 nmol/L [normal 0.00–0.02]; AChR modulating 100% loss [normal 0–20%]; striational 15,360 [normal <60]) and thyroglobulin, and anti-nuclear antibody.

On admission, the patient had memory impairment and signs of frontotemporal functional deficits, typical of FTD. Dysarthria rendered speech totally incomprehensi-

ble. He had mild eyelid ptosis bilaterally (without extraocular muscle weakness or diplopia) and was markedly quadriparetic (confined to wheelchair) with bilateral foot drop. Distal hand and foot muscles were moderately atrophic, and fasciculations were prominent in all limbs. Deep tendon reflexes were brisk in arms and legs (except hypoactive Achilles reflexes), and an extensor plantar response was evoked bilaterally. Superficial sensation was normal, and vibration sense was reduced slightly distally in the legs.

Due to the diagnosis of MG, the patient did not meet inclusion criteria for the Hadassah clinical trial in ALS with autologous enhanced mesenchymal stem cells (MSC-NTF, Brainstorm®, Petach Tikva; NCT01051882).<sup>2–4</sup> The Hadassah Ethics Committee issued a special license for treatment on a compassionate basis. MSC-NTF (prepared from the patient's bone marrow) were injected intrathecally ( $1.5 \times 10^6$  per kilogram of body weight) and at 24 sites along the biceps and triceps muscles of the right arm ( $1.5 \times 10^6$  per site). The intrathecal and intramuscular administration of the cells were chosen based on previous animal and clinical studies from our groups, which showed good migration of the intrathecally injected cells to the CNS<sup>4,5</sup> and amelioration of the “dying-back” phenomenon by intramuscularly injected MSC in early stages of ALS in the SOD mouse model (unpublished data and Dadon-Nachum et al.).<sup>6</sup> For the next 2 days the patient had a low-grade fever, headache, and was more confused, but at discharge, these problems had completely subsided. Treatment with azathioprine (125 mg/day) was discontinued 1 month before the injection and re-administered 30 days after the treatment. Pyridostigmine (60 mg 3 times daily) and low dose oral prednisolone (10 mg/day) were continued.

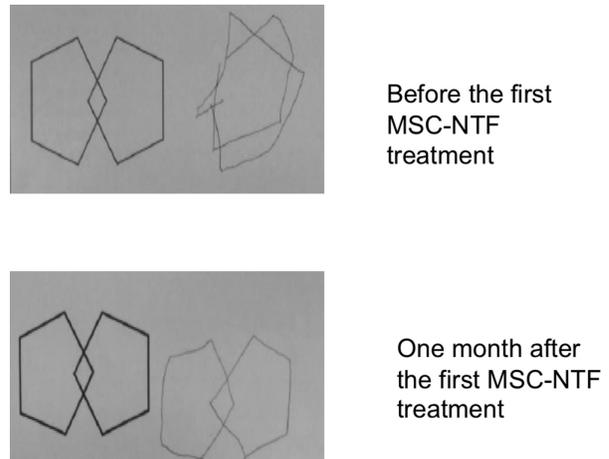
At 1 month after transplantation, the patient and his family reported significant improvement in cognition, speech, and muscle power. He was able to walk at least 20 meters without any support. The dysarthria improved to the extent he was able to clearly deliver a speech to an audience. ALS Functional Score Scale-Revised (ALSFRRS-R, performed at all time points by the same evaluator and confirmed by a second senior examiner) score rose from 36 to 44, and respiratory forced vital capacity (FVC) and cognitive function also improved significantly (Supplementary Table 1, which is available online, and Fig. 1).

At 5 months posttransplantation, due to progression of weakness and deterioration in cognition, a repeat injection of MSC-NTF, was performed. Adverse events following the second treatment included a transient confusional state, moderate fever, and a urinary tract infection. These symptoms subsided completely 3 days posttransplantation, and on his last examination 2 months after the second transplantation, all neurological functions had improved significantly. ALSFRS-R rose from 30 to 43, the quantitative MG score declined from 19 to 14, muscle power improved significantly, foot drop resolved partially, and the FVC increased (Supplementary Table 1). There was no detectable decrement in repetitive nerve stimulation (similarly to his pretransplantation test), but compound muscle action potentials (CMAPs) increased three-fold in the right arm.

The co-existence of ALS with MG is extremely rare.<sup>7-9</sup> The onset of motor neuropathy in our patient was accompanied by FTD and was preceded 2 years earlier by muscle weakness, attributed to MG. The patient's background of autoimmunity also included thyroiditis and the presence of anti-nuclear antibodies. It is conceivable in this context that the motor neuropathy and dementia could have had an autoimmune basis. This suggestion is supported by the modestly elevated cerebrospinal fluid protein level<sup>10</sup> (potentially attributable to his diabetes or intravenous immunoglobulin treatment) and the profile of organ-specific autoantibodies (including muscle AChR and striational antibody values commonly encountered with paraneoplastic neurological autoimmunity).<sup>11</sup> No cancer was found or imaged.

Stem cell therapy has been proposed and tried by others and us in various neurological diseases as a novel means for regeneration and neuroprotection.<sup>4,12-15</sup> Mesenchymal stem cells (MSC) are a particular contemporary focus of clinical research as a potential therapeutic agent due to their safety profile and the relative ease of isolation, culture, and expansion from bone marrow, even from adult patients with neurological disease. They appear to exert both immunomodulatory and neurotrophic/neuroprotective effects<sup>4,5,16-21</sup> and pose less risk for malignant transformation than embryonic stem cells. The outcomes of small pilot studies of therapy with MSC suggest a neurological stabilization in patients with ALS,<sup>4,13-15</sup> neuroregeneration of the optic tracts in at least 1 trial in progressive multiple sclerosis,<sup>12</sup> and beneficial clinical effects in multiple system atrophy.<sup>22</sup>

The course of the 2 neurological diseases in our patient and the response to the repeated treatments with MSC-NTF provide some unique observations. First, both cognitive and motor disabilities improved after the initial course of cell therapy; these benefits were reversed over the following months. The outcome of the second transplantation was even more remarkable, suggesting that repeated transplantation might be needed to maintain and enhance the clinical benefits of stem cell therapies in neurological diseases. The possibility of an evaluating physician treatment bias or "placebo effect" (although low in such severe degenerative diseases as ALS and contradicted by the electrophysiological and respiratory tests) cannot be excluded totally in such open studies. Second, the improvement in many



**FIGURE 1.** Figure copying (a test of visuospatial function) of the patient, before and 1 month after the first enhanced (neurotrophic factors producing) mesenchymal stem cell (MSC-NTF) transplantation.

parameters of the ALSFRS-R and the right arm CMAP (the site of MSC-NTF injection), advocate in favor of motor nerve function being more significantly affected by the treatment (than MG) and may indicate a neurotrophic effect. Laboratory and clinical improvements of this magnitude are not anticipated and are highly unusual in the natural course of ALS. Whether these beneficial effects were induced by transplantation of MSC-NTF cannot be proven, nor whether the neurological improvement was mediated by immunomodulating or neurotrophic effects or by promotion of neuroregeneration. The latter seems less likely, because improvement was evident early after transplantation, before neuronal repair or regeneration might be anticipated. If one assumes that the motor neuron dysfunction in our patient was due to ALS, rather than an immune-mediated process, the fact that the myasthenic manifestations benefitted less from the cell therapy favors a neurotrophic rather than an immunomodulatory basis for the observed clinical improvements. If the myasthenia, motor neuropathy and dementia all had an autoimmune basis, one could conclude that the benefit of cell therapy was attributable to the known immunomodulating properties of MSC.<sup>17,19</sup>

Additional data from our ongoing clinical trial in 24 patients with ALS will provide more concrete answers to the open questions and may reveal the mechanisms of action of MSC in neurodegenerative diseases such as ALS.

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## **PARADOXICAL WORSENING OF ANTI-MYELIN-ASSOCIATED GLYCOPROTEIN POLYNEUROPATHY FOLLOWING RITUXIMAB**

A recent controlled study has shown that rituximab, a monoclonal antibody directed against the surface marker CD20 that depletes circulating B cells, may be effective for patients with IgM paraproteinemic demyelinating polyneuropathy with antibodies to myelin-associated glycoprotein (MAG).<sup>1</sup> Anti-MAG neuropathy is an acquired demyelinating polyneuropathy that typically does not respond to other immunomodulatory therapies such as intravenous immunoglobulin. However, a potentially serious complication noted during treatment of Waldenström macroglobulinemia is a transient increase in IgM levels occurring in >40% of patients.<sup>2</sup> This is often associated with increased serum viscosity,<sup>3</sup> a phenomenon known as IgM flare.<sup>2</sup> IgM flare is believed to be the result of rituximab-induced B-cell lysis, with subsequent release of paraproteins that persist transiently, often for weeks. This report illustrates paradoxical worsening of peripheral neuropathy in a patient with anti-MAG polyneuropathy presumably due to IgM flare, an under-recognized association that could have dire consequences.

An 85-year-old man presented with advancing numbness in his feet and imbalance over 2 years. His examination was notable for stocking sensory loss to all modalities and mild sway with Romberg testing, but normal power. Electrodiagnostic study was consistent with distal acquired demyelinating symmetric neuropathy, with prolonged distal motor latencies in the upper and lower extremities (right common fibular 13.8 ms, normal <6.5 ms; right posterior tibial 10.7 ms, normal <7.0 ms; right ulnar 5.3 ms, normal <3.9 ms) and reductions of motor conduction velocities in the lower extremities (right common fibular 13 m/s in the distal leg, normal >36 m/s; no response with stimulation at the knee), but no motor conduction block or abnormal temporal dispersion. He was found to have a monoclonal IgM lambda spike by serum protein electrophoresis and an anti-MAG antibody titer of 1:12,800 (normal <1600; Athena Diagnostics). He began treatment with rituximab 375 mg/m<sup>2</sup> weekly for 4 infusions. After 2 infusions, he developed profound worsening of sensory ataxia and foot drop that markedly impaired ambulation along with a significant increase in paraprotein level, a 4-fold increase in MAG titer, and a 1.4-fold increase in total IgM (Table 1). The serologic parameters and his clinical status returned to baseline approximately 6 weeks after cessation of therapy, followed by modest clinical improvement.

Having treated at least 15 patients with anti-MAG neuropathy with rituximab, we have found that it is generally