**Intravenously Administered Autologous Bone Marrow and Adipose-Derived Stromal Cells in the Treatment of Alzheimer Disease: Case Studies**

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**ABSTRACT**

Alzheimer Disease (AD) has long been a debilitating problem that not only affects patients' quality of life but that of those who care about them.

We studied four Alzheimer disease patients with cognitive impairment who underwent adult harvested stromal vascular fraction (SVF) stem cell treatment at baseline, and then every 90 days for a total 180 days. St. Louis University Mental Status (SLUMS) cognitive performance test scores, SPECT (single-photon emission computed tomography), MRI (magnetic resonance imaging), and CSF (cerebrospinal fluid) protein analysis (including beta-amyloid and total tau protein) measurements were obtained at baseline, immediately following treatment, and 90 days after treatment ended (Day 216).

In two patients, improved SLUMS scores were obtained within the first 90 days of the initial treatment, and CSF levels of beta-amyloid and tau protein were decreased. Improvements in all of these domains were sustained across the group at day 216. The results of this pilot study suggest that administration of adult harvested stem cells may be associated with improvements in cognitive as well as biochemical measurements.

**Introduction**

Alzheimer disease (AD) is the most common form of dementia in all age groups, accounting for 60-70% of neurodegenerative cases. Life expectancy has increased around much of the world; therefore, the number of older people at risk for developing dementia has increased. AD is associated with disruption of neuronal function and a gradual deterioration of cognitive abilities.

AD progressively impairs episodic and semantic memory networks. The basal forebrain cholinergic neural system undergoes neurodegeneration. AD manifests initially with subtle progressive memory loss that eventually becomes severe and incapacitating. Behavioral deficits, including social withdrawal, aggression, depression, and hallucinations, may also be present.

Pathologically, AD is characterized by intracellular and extracellular formation of beta-amyloid plaques and neurofibrillary tangles within the brain and by cerebral atrophy. The cerebrospinal fluid (CSF)-based biomarkers beta-amyloid peptide (Aβ), phospho-tau (p-Tau) and total tau can aid in the diagnosis of AD. Studies performed with more than 70 participants showed sensitivity of 85-94% and a specificity of 54-95% in distinguishing AD from non-AD populations. The presence of p-Tau in the CSF has been found to discriminate AD from other dementias with sensitivities of 72-88% and specificities of 78-83%. The combination of all three biomarkers has been reported to have an average sensitivity and specificity of 85% and 90%, respectively. Aβ proteins occur within fatty membrane surrounding nerve cells, becoming chemically "sticky" and building up into plaques. These Aβ proteins clump together, causing delays in synapse firing, decreasing neuronal activity, and activating the immune system response, triggering inflammation. In healthy brains, Aβ is filtered from the nervous system by means of a transport system organized in orderly parallel strands, resembling railroad tracks. A second protein known as total tau helps the “tracks” to stay straight. Neurofibrillary tangles are made up of twisted strands of tau proteins, suggesting that the system is unable to transport or filter the Aβ.

Currently, AD-related neuronal cell death cannot be prevented. Recently, cell replacement therapy to compensate for neural decline has been examined. The therapeutic potential of adult stem cells, particularly mesenchymal stem cells (MSCs), based on their anti-inflammatory properties in experimental models of AD, seem promising. MSCs are stromal cells that can be readily harvested from numerous tissues, including bone marrow (BM), the stromal vascular fraction (SVF) of adipose tissue, and cord blood. All of these cell types release exosomes, which decrease Aβ in the central nervous system.

Finding a means of decreasing Aβ and p-Tau appears to be one way to improve the AD patient’s neurologic signs and symptoms. Neurofibrillary tangles are directly related to the level of Aβ and tau in the brain, and there appears to be a direct relationship between the amount of Aβ and the amount of tau in the brain.

Adipose-derived stem cells (ASCs) from the SVF of adipose tissue may be clinically suitable because they are easily accessible, highly proliferating in vitro, and can undergo differentiation into multiple cell lineages. Additionally, implantation of human ASCs (hASCs) show few side effects such as oncogenicity or immune rejection, suggesting that autologous hASCs pass through the blood brain barrier and that this treatment is safe in humans.

Administration of bone marrow-derived mesenchymal stem cells (BM-MSCs) is beneficial in animal models for several neurodegenerative diseases such as Parkinson disease, experimental autoimmune encephalomyelitis, and amyotrophic lateral sclerosis. BM-MSC transplantation has been suggested as a potential therapeutic approach for Alzheimer disease, but the mechanism of action has not yet been ascertained.

Given promising results from animal studies, using human MSC from either bone marrow or adipose tissue may produce beneficial effects in humans with AD. In this case study, we demonstrate cognitive benefits from intravenous administration of MSCs for select individuals with minimal cognitive impairment from AD. Following a short intervention, positive changes were observed in the SLUMS (St. Louis University Mental Status) examination. We theorize that improvements may be linked with a suppressed cortical inflammatory response, improved transport, or filtration of amyloid and tau from the brain/spinal fluid.

The role of light in biological processes includes vitamin
D conversion and photoactivation when skin is exposed to sunlight. Of course, plants use photosynthesis to make food. Less well known is the effect of photomodulation on many cellular processes involving flavins, cytokines, iron-sulfur complexes, hemes, transition-metal ligand bonds, and enzymes. We used photoactivation by exposure of platelet-rich plasma (PRP) and stem cells to light-emitting-diode (LED) light. Important considerations include wavelength; duration, frequency, and intensity of exposure; and the presence or absence of cofactors, enzymes, and other catalysts. The choice is critical because photomodulation can also have a negative or deleterious effect on the cells. Adistem has discovered five light frequencies that activate stem cells in vitro, and two frequencies that will inhibit activation.\(^{17}\) When ASCs were photoactivated for 20 minutes with the Adilight device, they showed increased release of signaling factors such as integrins, VEGFs, thymosin beta 4, and interleukin 1 receptor antagonists. These substances are instrumental in promoting healing.\(^{18,19}\)

### Materials and Methods

This case study was designed for MSC intervention using a sample of convenience. No randomization or placebo control arm was performed. The study was conducted at Precise Clinical Research Solutions (Topeka, Kansas, USA). Institutional Review Board approval was given by International Cell Surgical Society, (IRB Approval Number: ICSS-2013-90). Inclusion and exclusion criteria, and primary and secondary end-points are shown in Table 1. Our study utilized adipose-derived stromal cells and bone marrow stromal cells (Figure 1).

### Table 1. Individual Effects of the MSC Intervention in Alzheimer Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Duration of Disease</th>
<th>SLUMS</th>
<th>Beta Amyloid</th>
<th>Tau</th>
<th>Level of Impairment</th>
</tr>
</thead>
<tbody>
<tr>
<td>01-000</td>
<td>79</td>
<td>M</td>
<td>18 mo.</td>
<td>15</td>
<td>3418</td>
<td>1369</td>
<td>577 911</td>
</tr>
<tr>
<td>01-001</td>
<td>64</td>
<td>F</td>
<td>2 years</td>
<td>17</td>
<td>1102</td>
<td>1796</td>
<td>41 243</td>
</tr>
<tr>
<td>01-002</td>
<td>79</td>
<td>M</td>
<td>2.5 years</td>
<td>20</td>
<td>1150</td>
<td>806</td>
<td>715 529</td>
</tr>
<tr>
<td>01-003</td>
<td>71</td>
<td>M</td>
<td>2 years</td>
<td>27</td>
<td>1057</td>
<td>118</td>
<td>566 108</td>
</tr>
</tbody>
</table>

### Figure 1. Case Study Protocol

Four patients, three male, one female, aged 64-79, with SLUMS scores of at least 17 and neurologist-diagnosed AD, were recruited and approved for this study. Patients and caregivers gave informed consent. AD was diagnosed by a positive SPECT (single-photon emission computed tomography) scan of the brain and SLUMS neurological testing (see Table 1). The SPECT scan was completed at rest followed by stress. At rest included placing the patient in a dark room for 10 minutes prior to scanning. The stress scan included having patient complete the psychological Stroop test then immediate SPECT scanning. SLUMS tests were performed by a trained staff member to ensure repeatability and reliability. SLUMS was chosen over other comparable testing methods such as the MMSE (Mini-Mental Status Exam) due to its supposed superior reliability in detecting cognitive impairment.\(^{20}\) It was also preferred by trained staff members for administering serial tests. CSF proteins including Aβ and total tau protein were measured. These proteins increase as the disease progresses, and reduction in these values should be an indicator of disease regression. CSF P-tau, now available from Athena Diagnostics Laboratories, was not available at the time this study was started.

Clinical measurements were recorded at baseline, and every 90 days for 216 days total (see Figure 1). Each patient then underwent infusion of adult harvested SVF, as described in the SVF phase Ib clinical trial regarding the safety of ASCs in idiopathic fibrosis,\(^{21}\) followed by treatment every three months for a total of three treatments (See Figure 1). Within two weeks of the final treatment, SPECT imaging and CSF protein analysis were repeated. Serial mental status examinations (SLUMS) were also performed between each SVF treatment (see Table 1 and Figure 1).

SVF for treatments was derived from mini-liposuction with collection of 60 g of fat plus bone marrow aspirate with collection of 60 cc of bone marrow. The adipose aspirate was lysed using lecithin, and centrifuged and filtered twice. The bone marrow aspirate was centrifuged and filtered twice. The SVF was removed from both adipose and hematopoietic aspirates and mixed with PRP. The mixture of SVF from adipose and hematopoietic aspirates and PRP was activated for 20 minutes using red, yellow, and green wavelengths of light following the Adistem technique.\(^ {17}\) The SVF-PRP mixture was added to 500 cc of normal saline, and the saline was administered intravenously via peripheral intravenous infusion. The SVF mixture was given over a two-hour interval. This procedure was performed every three months for a total of three treatments.

### Results

No adverse responses were observed in the four patients. Two patients (01-002 and 01-003) completed the entire protocol with positive changes in all measurements (See Table 1). In patient 01-002, there was a 30% improvement in SLUMS, a 29.9% decrease in Aβ, and a 26% decrease in tau. In patient 01-003, there was a 35% improvement in SLUMS, an 88.8% decrease in Aβ, and an 80.9% decrease in tau. Both patients showed specific improvement in the attention and working memory portion of the SLUMS, with immediate positive functional results after the first treatment. Patient 01-002 was able to tie his shoes 48 hours after the initial treatment and maintain attention during an entire football game, both functions he had lost prior to treatment. Patient 01-003 was able to easily navigate during shopping without getting lost. These patients had improved SLUMS scores within 90 days of treatment initiation as well as sustained improvements at 216 days post treatment.

Due to caregiver burden, patient 01-001 (female; see Table 1) dropped out of the study after the second treatment (Day
There were increases in both Aβ (62.9%) and tau (493%) in this patient, and a worsening of the SLUMS score (by 11.8%).

Patient 01-000 completed the entire protocol and had a 4.3% decrease in Aβ and a 7.24% decrease in tau. However, this patient also had a 20% decrease in SLUMS score.

In very early stages of AD, nuclear medicine imaging modalities of the brain revealed losses of gray matter in the hippocampal areas and hypoperfusion in the posterior cingulate cortex and precuneus. These early findings have been predictive of the conversion from mild cognitive impairment (MCI) to AD.22

There has been some controversy over which nuclear medicine brain scan modality is superior for evaluating patients with AD. Silverman found positron emission tomography (PET) to be superior to SPECT in the early diagnosis of AD because of PET’s higher sensitivity and higher spatial resolution. The 18F-FDG PET does offer some advantages over SPECT for detecting abnormalities in a brain affected by AD. However, SPECT has some advantages. SPECT is less expensive and more accessible, being found in nearly all hospitals. The affordability and ease of access could lead to a large increase in the number of cases that could immediately be studied with this technique for early assessment of neurodementia.23

Each patient was given 555 to 1110 MBq (15-30 mCi) of Ceretec (technetium 99M hexamethyl propylene amine oxime [HMPAO]), and SPECT imaging of the brain was performed following the American College of Radiology (ACR) nuclear medicine brain scan protocol,24 and a rest baseline scan was obtained. At a minimum of 30 hours later, five times the 6-hour half-life of technetium 99M, a second brain scan was completed. The subject was given Ceretec intravenously, and a Stroop Color and Word test was performed to stimulate cerebral activity, then a second (stress) SPECT brain scan was performed. This two-part scan is analogous to the nuclear medicine rest/stress thallium cardiac scan.

The SPECT imaging was then completed at the 9-month interval, 3 months after the third treatment.

Subject 01-001, as evidenced by the brain SPECT scans before (Figure 2) and after (Figure 3) therapy did not experience any change. This subject was considered minimal to moderately cognitively impaired, and this subject’s CSF protein markers increased. It is hypothesized that this patient was already too far advanced to see any improvement with therapy, and in fact, there was an obvious worsening.

Subject 01-003 SPECT brain imaging before (Figure 4) and after (Figure 5) therapy, on the other hand, showed a modest improvement in overall brain perfusion and some mild improvement in hippocampal perfusion. This subject was assessed as minimally cognitively impaired and also had earlier intervention, a possible explanation for the subject’s showing favorable results to therapy, both clinically and on diagnostic testing (as observed by an increase from 20 to 27 on the SLUMS test, improved SPECT imaging, and decreasing CSF protein markers.)
In addition, behavioral recovery following cerebral ischemia in rats after transplant of BM-MSC was associated with elevated brain-derived neurotrophic factor (BDNF), NT-3, and VEGF levels. After hASCs transplantation, AD mice models showed increased VEGF, GDNF, NT-3, and anti-inflammatory cytokine IL-10, while IL-1β remained unchanged. Furthermore, MSCs used in cerebral infarction model rats affect cytokine release by up-regulating IL-10 and down-regulating TNFa. These findings suggest that pro-inflammatory cytokines negatively impact neurogenesis, whereas anti-inflammatory cytokines exert a positive effect. This suppression of inflammation may offer a partial explanation as to why two of our four patients had an improvement in their mental status scoring after three treatments.

Considering possible explanations for the decrease in CSF protein markers Aβ and tau and the improved cognition in two of our four patients, the innate immune system is the first line of defense against a wide range of pathogens and tissue injuries, triggering inflammation through activation of microglia and macrophages. As such, using BM-MSC as an intervention in order to activate microglia may serve to replicate the body’s natural defense system during specific disease processes. Many studies have shown that microglia are attracted to and surround senile plaques both in human AD samples and in rodent transgenic models that develop AD-related disease. However, the exact role of inflammation underlying the pathogenesis of AD remains to be elucidated.

Some studies have also demonstrated that Aβ activates microglia to produce cytokines and neurotoxins, hence promoting neurodegeneration. In contrast, others have suggested that microglia secrete neurotrophic agents and eliminate toxic Aβ by phagocytosis. The effects of BM-MSC transplantation in an ischemia mouse model were mediated through microglia and macrophage activation, and these immune cells were “alternatively activated” in order to provide an anti-inflammatory and repair role.

Many studies support the idea that bone marrow-derived cells are able to cross the blood-brain barrier and differentiate into microglia, or induce endogenous microglia/macrophage activation. A previous report also showed that BM-MSCs could increase microglial activation and reduce Aβ deposits in an acutely induced AD model.

The effect of BM-MSCs on reducing Aβ accumulation is likely attributable to restoration or enhancement of the Aβ-clearance pathway via microglial cells. Indeed, stimulation of the immune system in AD rodent models reduced Aβ burden. Aβ is phagocytosed by microglia and delivered to the lysosome, where it is subsequently degraded. However, primary culture of mouse microglia indicates that these resident immune cells of the central nervous system need to be activated prior to acquiring the ability to clear Aβ from the brain. These newly recruited microglial cells are specifically attracted to the Aβ 40/42 isoforms in vivo, and they participate in elimination of these proteins by phagocytosis. Activated microglia were able to phagocytose Aβ, and their selective ablation resulted in an increased amyloid burden. Similarly, C-C chemokine receptor-2 (CCR2) gene deficiency was found to impair microglia recruitment and increase amyloid deposits in APP mice. Also, decreased microglial cell recruitment, by genetic ablation of the TLR2 receptor in a PS1/APP model, increased Aβ42 levels and accelerated memory impairment. Theoretically, BM-MSCs treatment either prevented the synthesis of Aβ peptide, or enhanced clearance of existing Aβ deposits through microglia.

Figure 5. SPECT Imaging 01-003, After Therapy. Mild to moderate hippocampal atrophy. Some improvement in cerebellar atrophy. Mild to moderate temporal and fronto-parietal atrophy. Mild improvement in diffuse cerebral brain perfusion (increasing green).

Discussion

Our study design and sample of convenience provides limited results. Individuals received the same dosage or quantity of SVF (60 cc of BM aspirate and 60 g of adipose), regardless of their body mass. Also, the ideal or required duration between treatments is unknown. Further, the appropriate treatment frequency is unclear. Future studies should consider functional MRI simultaneously with neuropsychological tests to more fully assess neural changes. Additionally, live-dead cell and nano-particle analysis of SVF specimens is desirable.

Two patients were more debilitated cognitively at the onset of the study and showed less favorable results, although promising findings occurred with the two patients with less cognitive impairment. Post-treatment measurements revealed that these two patients demonstrated sustained decrease in CSF Aβ and total tau protein markers and improved SLUMS scores from baseline. Future studies should explore the relationship in Aβ, tau and SLUMS after each treatment interval, which was not possible from our study design.

There are several potential reasons for the decreased levels of Aβ and tau proteins as well as the improved SLUMS scores. We believe that suppression of inflammation, accelerated degradation of Aβ protein, and stem cell-mediated activation of microglia and neurons could all possibly be implicated. Interactions between them might also be important.

According to Akiyama et al., inflammation clearly occurs in pathologically vulnerable regions of the Alzheimer disease brain. Damaged neurons and neurites, insoluble Aβ peptide deposits, and neurofibriillary tangles provide obvious stimuli for inflammation. MSC therapy could possibly result in suppression of underlying inflammation and have favorable influence upon damaged neurons and neurites. This anti-inflammatory response coupled with the decrease in the Aβ and tau proteins is associated with results of improved cognition, as reflected in improved SLUMS scores in our study. ASCs produce and secrete cytokines/growth factors that can suppress the inflammatory response. These include many growth factors such as vascular endothelial growth factor (VEGF), glial cell-line-derived neurotrophic factor (GDNF), neurotrophin-3 (NT-3), nerve growth factor (NGF), and basic fibroblast growth factor (FGF), which regulate pro-inflammatory cytokine release.
activation. Consequently, activation of microglia with BM-MSC may prevent Aβ accumulation or reduce Aβ deposits in the central nervous system.12,15

We observed a decrease in the Aβ and tau protein markers in the spinal fluid after three treatments for two patients. Neprilysin (NEP) is the most important Aβ degrading enzyme in the brain8,31 and is thought to be capable of degrading both intra- and extracellular accumulation of Aβ protein. It is hypothesized that a similar relationship between NEP and tau also exists. The fact that hASCs secrete exosomes for enzymatically active NEP suggests therapeutic relevance for AD,19 potentially explaining some of our results.

In Huntington disease, hASCs attenuated the loss of striatal neurons and reduced the Huntingtoning aggregates leading to improved rotarod performance and survival.12 Intravenously transplanted hASCs have been shown to pass the blood-brain barrier and migrate into the brains of an AD model in mice. Cognitive and neural structural improvements were observed within 2–8 weeks after transplantation into the AD mouse model.16

We suggest that a larger randomized clinical trial could explore these relationships and examine the overall benefit in functional performance for those suffering from AD. If each individual had an initial harvesting procedure of their hematopoietic and mesenchymal stem cells with cryopreservation of the cells, then patients could have periodic treatments every month for a prolonged period.

Conclusion

Half of a small sample of Alzheimer disease patients with mild cognitive impairment showed measurable benefit in biochemical markers as well as in cognitive function after treatment with autologous stem cells. Experimental work in animals suggests several mechanisms through which stem cells could affect the pathophysiology of the disease.


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REFERENCES


