Wharton’s jelly-derived mesenchymal stem cells in the treatment of four patients with alopecia areata

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Abstract

**Background.** Alopecia areata (AA) is the second most common cause of non-scarring alopecia. Little is known on the etiopathogenesis of AA. It is considered an autoimmune disease, with T lymphocytes and antibodies directed against hair follicle structures. Topical and systemic therapies are used for the treatment of AA, but none of the therapies used to date have a permanent therapeutic effect.

**Objectives.** To evaluate the efficacy and safety of AA treatment through a single intradermal injection of a suspension of allogeneic MSCs extracted from Wharton’s jelly (WJ-MSCs) into the alopecia foci.

**Material and methods.** The study involved 4 AA patients who underwent experimental therapy with a suspension of WJ-MSCs. The AA intensity was measured using the SALT score. This measure was performed 3 times during treatment: 1st measure (SALT₀) prior to treatment; 2nd measure (SALT₁) 12 weeks after the treatment; and 3rd measure (SALT₂) 24 weeks after the treatment. Furthermore, during each follow-up visit (6, 12, 18, and 24 weeks after the administration of WJ-MSCs) the patient’s general condition (physical examination) and local condition were assessed, their mood was evaluated, and a photo of the scalp was taken.

**Results.** Hair regrowth was observed in all patients by an average of 67% at the sites where the cell suspension was administered. In all cases, we observed greater dynamics of hair regrowth in the first 3 months after the treatment, with an average increase of 52.2%, compared to the following 3 months, with an average of 32%.

**Conclusions.** The results of the applied intradermal injections of an allogeneic WJ-MSC suspension were positive with hair growth observed in all participants and the therapy was found to be safe, with no side effects.

**Key words:** alopecia areata, Wharton’s jelly, mesenchymal stem cells, MSC, WJ-MSC
**Background**

Alopecia areata (AA) is the 2\textsuperscript{nd} most common cause of non-scarring alopecia after androgenic alopecia (AGA).\textsuperscript{1} The prevalence is estimated at 2% and has increased tenfold since the 1970s when it affected 0.1–0.2% of the population.\textsuperscript{2} It equally affects both genders.\textsuperscript{3} The severity of the disease varies widely, from a single focus of a few centimeters in diameter to extensive hair loss in all or some regions of the body, including the scalp, eyebrows and eyelashes, and the rest of the body. In some patients, nail dystrophy has also been observed. The disease leads to a significant deterioration of patients’ quality of life.\textsuperscript{4} There are several clinical forms of the disease: focal hair loss (the most common), diffuse alopecia, ophiasis, total alopecia (total loss of scalp hair), and alopecia universalis (loss of all body hair).\textsuperscript{5} Alopecia focal lesions are not usually accompanied by subjective complaints, although 14% of patients report itching or burning. The course of the disease is unpredictable, with 90% of patients experiencing recurrences after the 1\textsuperscript{st} episode in the first 5 years, and some showing spontaneous remissions.\textsuperscript{6}

Little is known on the AA etiopathogenesis. It is considered an autoimmune disease, with T lymphocytes and antibodies directed against hair follicle structures.\textsuperscript{6} Indeed, histopathological examination of the skin reveals abundant perifollicular lymphocytic infiltrates,\textsuperscript{7,8} and this infiltration mainly affects follicles in the anagen phase.\textsuperscript{6}

The autoantigenic epitopes are thought to include melanin and melanin-related proteins, and keratinocyte antigens. In the pathogenesis of the disease, the loss of immune privilege is also important. Healthy hair follicles are classified as tissues with immune privilege, i.e., they do not induce an aggressive immune response when transplanted using an allogeneic regimen. The immunological privilege in healthy hair follicles is attributed to the reduced expression of class I major histocompatibility complex (MHC I) antigens on the keratinocytes, a lack of expression of these antigens on melanocytes and a lack of antigen-presenting cells in the lower part of the hair follicle, together with a small number of immunocompetent cells and the presence of immunosuppressive factors.\textsuperscript{9,10}

Genome-wide association studies (GWAS) have identified various genes associated with the pathogenesis of AA (e.g., \textit{IL2/IL21, IL2RA, CTLA4, ULBP3, and STX17}).\textsuperscript{11} Genetic factors are also involved in the pathogenesis of AA, as the disease is also seen in first-degree relatives and monozygotic twins.\textsuperscript{12,13} In 16% of patients, other autoimmune diseases are also present, most often vitiligo and autoimmune thyroid diseases.\textsuperscript{1,5,12}

Topical and systemic therapies are used for the treatment of AA. In topical therapy, the following medications are used: glucocorticoids in the form of external preparations and injections into the affected skin, minoxidil, contact immunotherapy (e.g., diphenylcyclopentenone and cygnolin), and drugs administered systematically (e.g., glucocorticoids, methotrexate, cyclosporine, azathioprine, and sulfasalazine).\textsuperscript{1,6,14} Moreover, treatment also includes UV phototherapy in the form of excimer laser and psoralen and ultraviolet A (PUVA) therapy,\textsuperscript{1} as well as superficial cryotherapy.\textsuperscript{15,16} However, none of the therapies used to date have a permanent therapeutic effect.

The risk of side effects of the commonly used therapies significantly reduces their use. New therapeutic alternatives are therefore constantly being sought. Recently, the efficacy of Janus kinase inhibitors (applied topically and systematically), prostaglandin analogues, statins, platelet-rich plasma, and stem cells has been reported. The medical literature contains limited descriptions of experimental therapies using autologous mesenchymal stem cells (MSCs) extracted from the patient’s adipose tissue for the treatment of AA.\textsuperscript{9,17}

Stem cells can be divided into 3 different types: embryonic stem cells (ESCs), adult stem cells (ASCs) and induced pluripotent stem cells (iPSCs). The use of ESCs raises ethical controversies that do not apply to tissue-derived stem cells, e.g., MSCs.\textsuperscript{18} In cell therapies, MSCs are used most commonly. The MSCs can be extracted from various tissues: bone marrow, adipose tissue, umbilical cord blood, Wharton’s jelly (allogeneic MSCs extracted from Wharton’s jelly – WJ-MSCs), and the amniotic membrane.\textsuperscript{18} Cells extracted from bone marrow, adipose tissue and Wharton’s jelly (WJ) are of most practical importance.\textsuperscript{17,19,20} It is worth noting that the extraction of MSCs from WJ is noninvasive.\textsuperscript{21} In 2006, the International Society for Cellular Therapy (ISCT) defined the minimum criteria required for a cell to qualify as an MSC: (1) adhesion to plastic; (2) expression of CD73, CD90 and CD105 surface antigens in the absence of hematopoietic antigens CD34, CD45, CD14 or CD11b, CD79a or CD19, and human leukocyte antigen – DR isotype (HLA DR) surface antigens; and (3) the ability to differentiate into osteoblasts, adipocytes or chondrocytes in vitro.\textsuperscript{22}

Mesenchymal stem cells possess immunomodulatory properties.\textsuperscript{18} It has been demonstrated that MSCs reduce the proliferative properties of T and B lymphocytes and NK cells, secrete numerous paracrine factors including anti-inflammatory cytokines, and have the ability to migrate toward the site of damage. The MSCs change the secretion profile of immune cells towards anti-inflammatory cytokines and contribute to an increase in the regulatory T lymphocyte population.\textsuperscript{9,22} The most important cytokine secreted by MSCs that modulate T lymphocytes is interleukin 6 (IL-6).\textsuperscript{18}

Due to the immunological properties of MSCs harnessed from adult and fetal tissues they can be administered through an allogeneic regimen without the need to test the recipient and donor’s HLA systems and initiate immunosuppressive therapy in the recipient. Unlike embryonic cells, MSCs have no tumorigenic potential.\textsuperscript{23} In the European Union, cell therapy products that include MSCs have been considered medications since
2003. They are used to treat shin ulcers and hematological, orthopedic, urological, and gastroenterological disorders.

Previous attempts to use autologous MSCs for the treatment of alopecia have demonstrated the effectiveness and safety of this form of treatment. So far, allogeneic transplantation of WJ-MSCs has not been used in AA treatment. Due to the limited efficacy of the currently applied methods for the treatment of AA, it is important to search for new forms of therapy.

Objectives

The aim of this study was to evaluate the efficacy and safety of AA treatment through a single intradermal injection of a WJ-MSC suspension into the alopecia foci. The primary endpoint was the percentage change in the Severity of Alopecia Tool (SALT) score during treatment.

Material and methods

This experimental study was conducted with patients undergoing treatment at the Regional Specialist Hospital in Wrocław, Poland, and was performed in collaboration with the Polish Stem Cell Bank (Polski Bank Komórek Macierzystych – PBKM). The study protocol was approved by the local bioethics committee of the Research and Development Centre of the Regional Specialist Hospital in Wrocław (approval No. KB 01/2019) and all procedures performed in the study were carried out in accordance with the ethical standards of the Helsinki Declaration. All patients were informed in detail about the purpose and methods of the study, as well as the potential benefits and risks of therapy. All participants provided written informed consent to participate in the study.

Patients

The study involved 4 AA patients who underwent experimental therapy with a suspension of WJ-MSCs. The patient population included 3 men aged 36, 43 and 49 years and 1 woman aged 57 years. The duration of the disease ranged from 2 to 9 years (mean of 5 years). Three or more AA foci were observed in each patient (Table 1). In the past, all of the patients had undergone the following therapies: systemic and topical glucocorticoids, topical minoxidil, cryotherapy, and phototherapy (UVB 311 nm). One patient was treated with contact immunotherapy (diphenylcyclopropenone). None of these methods resulted in complete hair regrowth.

The patients did not suffer from any skin diseases or systemic diseases other than alopecia. For 6 weeks prior to the study, the patients did not take any medications. In all patients with AA, additional tests were performed prior to the initiation of therapy, which revealed C-reactive protein (CRP) values ≤5 mg/L, negative antinuclear antibodies (ANA) panel results, and no hepatitis C virus (HCV), hepatitis B virus (HBV) and human immunodeficiency virus (HIV) infections. On the day of administration of the WJ-MSC suspension, no clinical signs of other active infections were found.

Preparation of the Wharton’s jelly mesenchymal stem cell suspension

All umbilical cord (UC) collections were performed after obtaining informed consent of the parents. The UCs samples were collected after natural delivery or caesarean sections. The fragment of the UC as long as 20–30 cm was placed in a sterile container into with 0.9% natrium chloratum solution (Fresenius Kabi, Bad Homburg vor der Höhe, Germany) and transported in protective boxes to the laboratory. The transport conditions were monitored and UC tissue was processed within 72 h after delivery. Qualification of UC tissue requires providing complete responses to a medical questionnaire and submitting by donor-mother a peripheral blood sample for infectious agents testing for HBV, HCV, HIV, cytomegalovirus (CMV), and syphilis. All steps of manufacturing were performed in accordance the principles of Good Manufacturing Practice (GMP) and Good Laboratory Practice (GLP). Umbilical cord fragment was removed from transportation container and placed into a new container with 0.9% natrium chloridum solution (Fresenius Kabi) supplemented with 1% Antibiotic Antimycotic (Thermo Fisher Scientific/Gibco, Waltham, USA). After washing, the fragment was dissected into 2 cm fragments, put on 90 mm Petri dish (Medlab Products Sp. z o.o., Raszyn, Poland) and cut along with surgical blade; then, the arteries and the vein were removed with tweezers. After all blood vessels were removed, WJ

| Patient/patients’ initials | Gender | Age [years] | Duration of the disease [years] | Number of foci of alopecia |
|---------------------------|--------|-------------|-------------------------------|---------------------------|
| 1/BR                      | female | 57          | 9                             | 3                         |
| 2/PT                      | male   | 43          | 6                             | 4                         |
| 3/DH                      | male   | 36          | 2                             | 4                         |
| 4/PI                      | male   | 49          | 3                             | 9                         |
tissue was minced into 1–2 mm³ scraps and placed in xeno-
free, serum-free media into culture flasks for primary explants cultures development. Flasks were incubated in optimal conditions. After 14 days in culture, the tissues were removed from culture and the adherent cells were trypsinized using TrypLE® Select 1x (Life Technologies, Carlsbad, USA) and passed into new flasks for further expansion. The criteria for defining multipotent MSCs were established in 2006 by ISCT. They are based on cells adherence to plastic, presence of specific antigens like CD90, CD73 and CD105, together with the absence of hematopoietic and immune system markers (CD45, CD34, CD14 or CD11b, CD79a, CD19) and HLA-DR surface antigens, as well as conferring stem cell identity to differentiate into osteoblasts, chondrocytes and adipocytes. The pharmaceutical form of the product is a frozen preparation of cells in a cryoprotective liquid. The administration and dosage form of the treatment is a thawed suspension of WJ-MSCs with the phenotype CD73(+), CD90(+), CD105(+).

Procedure

For each patient, a single WJ-MSC suspension was administered to several alopecia foci with a total area of about 15 cm² in the form of intradermal injections. For 1 treatment, 2 mL of the suspension was used, which contained 5 × 10⁶ WJ-MSCs of phenotype CD73(+), CD90(+), CD105(+) suspended in a saline solution. Injections were performed into every 0.5 cm of skin surface. For each injection site, 0.01 mL of the suspension was administered. A needle (32 G, 0.23 × 6 mm) was used to administer the treatment. The scalp was anesthetized locally with EMLA cream (5% lidocaine) (Aspen Pharmacare, Durban, South Africa) 1 h before the treatment.

On the day of WJ-MSCs administration, patients were hospitalized for 1 day. Before the procedure, the severity of alopecia was assessed using the SALT score (SALT₀), the patient’s general condition (physical examination) and well-being were assessed, and photographs of scalp skin were taken. Patients’ general and local condition and well-being were reassessed 24 h after the procedure.

Evaluation of disease severity and effect of therapy

The AA intensity was measured with the SALT score. For this purpose, the scalp was divided into 4 areas where hair loss was assessed (40% vertex, 18% right side, 18% left side, 24% posterior); then, the percentage of hair loss over the entire scalp was calculated. This measure was performed 3 times during treatment: SALT₀ prior to treatment; SALT₁₂ 12 weeks after the treatment; and SALT₂₄ 24 weeks after the treatment; then, the observations concluded.

To evaluate the effects of therapy, we calculated the difference in alopecia surface area before and after application of the WJ-MSC suspension in the 12th and 24th weeks, assuming that the condition before treatment commencement was 100%, according to the following formula:

\[
(A - B/A) \times 100\% = I \text{ or } D,
\]

where A indicates the percentage of baldness before treatment, B is the percentage of baldness after treatment and I (improvement) indicates the amount of hair regrowth. If increased hair loss was observed after treatment, the condition was indicated by D (deterioration). Regrowth observed 12 weeks following treatment was marked as I₁₂ and regrowth after 24 weeks was marked as I₂₄ (in both cases taking the pretreatment condition as baseline). Additionally, in order to examine the dynamics of hair regrowth in relation to the time that had passed since treatment, the I₂₄/I₁₂ regrowth was determined 24 weeks after the treatment, taking the 12-week condition as baseline.

The efficacy of therapy was assessed for the whole surface of the scalp and for the following individual areas: vertex, right side, left side, and posterior (Table 2). Hair regrowth or loss in individual areas was determined according to the formula:

\[
(I \text{ or } D)_{24\text{vertex}}.
\]

Follow-up visits

Follow-up visits took place on an outpatient basis 6, 12, 18, and 24 weeks after the WJ-MSCs administration. During each follow-up visit, the patient’s general condition (physical examination), mental wellbeing and topical conditions were assessed, and a photo of the scalp was taken. When assessing the condition of the topical local site, particular attention was paid to symptoms that could indicate adverse effects of intradermal cell administration, such as redness or swelling. The SALT assessments were performed 12 weeks (SALT₁₂) and 24 weeks SALT₂₄ following treatment. Hair regrowth or loss were calculated and expressed as (I or D)₁₂, (I or D)₂₄ and (I or D)₂₄/₁₂.

Results

On the day of the administration of the WJ-MSC suspension, the SALT₀ score for the 4 patients (Table 2) was 26.4% for patient 1 (BR), 23.4% for patient 2 (PT), 19.4% for patient 3 (DH), and 40% for patient 4 (PI). The average SALT₀ score for all patients was 27.3% of hair loss.

In all patients, hair regrowth was observed at the sites of cell suspension administration 12 weeks after the procedure, together with a decrease in the SALT₁₂ value. The SALT₁₂ values for patients 1, 2, 3, and 4 were 17.6%, 7.2%, 12.2%, and 22.5%, respectively, with an average SALT₁₂ score of 14.9%.

Further improvement was observed in the 24th week. Hair loss after 24 weeks, measured using the SALT₂₄ score, was lower than that observed after 12 weeks (SALT₁₂), and
was 16.8% for patient 1, 4.8% for patient 2, 9% for patient 3, and 18.2% for patient 4. The average SALT score was 12.2% (Table 2).

Regrowth (I_{12}) of the scalp where the procedure was carried out was between 53% and 80%, with values of 80%, 79%, 53%, and 54% for patients 1, 2, 3, and 4, respectively (Table 2). None of the observed cases achieved 100% hair regrowth. The mean hair regrowth in the injected sites (I_{34}) was 67%.

All the patients were found to present with more hair regrowth after the first 12 weeks (I_{12}) than in the following 12 weeks (I_{24:12}) at the site where the intradermal WJ-MSC suspension was administered (Table 2). For patient 1, the I_{12} was 60% for the first 12 weeks and the I_{24:12} was 50% for the following 12 weeks; patient 2 had an I_{12} of 69% and I_{24:12} of 33%; patient 3 had values of 37% and 26%, respectively; and patient 4 had an I_{12} of 43% and I_{24:12} of 19% (Table 2). The mean I_{12} and I_{24:12} were 52.2% and 32%, respectively.

A noteworthy case was patient 1 (BR), who received posterior, left- and right-side injections, and by the 12th week was found to have hair loss in the vertex area which was not treated with injections of the WJ-MSC suspension. This deterioration was expressed by the symbol (D_{vertex}), and in the 12th week of therapy, the deterioration (D_{24:12vertex}) was 8% and increased to 12% after the following 12 weeks of observation (D_{24vertex}). The rate of hair loss increased and the D_{24:12vertex} score was 33% (Table 2). No hair loss was found in the remaining area of the scalp, while hair regrowth was observed in the injected sites (Table 2).

In patients 2, 3 and 4, no new foci of alopecia were found during the 24-week observation period. Improvement was observed in all treated areas: vertex, posterior, left side, and right side. Detailed results are presented in Table 2.

During the 24-week observation period, patients did not present any abnormalities in the physical examination. No local side effects (rash or swelling) at the site of intradermal injection of the WJ-MSC suspension were found in any patients. All patients reported good general health state and did not report any subjective symptoms.

Figures 1, 2, 3 present the condition before the treatment and results of the therapy in patient 1.

### Discussion

The introduction of stem cell-based therapies to repair and regenerate various tissues and organs offers innovative therapeutic solutions. Mesenchymal stem cells play an important role in the production of active agents for tissue regeneration, affecting the proliferation and migration

| Parameter | Patient 1 (BR) | Patient 2 (PT) | Patient 3 (DH) | Patient 4 (PI) |
|-----------|---------------|----------------|----------------|---------------|
| Vertex SALT | 0% 8% 12% | 0% 0% 0% | 12% 8% 6% | 28% 16% 14% |
| Vertex improvement I_{12}, I_{34} | – 0 0 | – – – | – 33% 50% | – 42.8% 50% |
| Vertex deterioration D_{12}, D_{34} | – 8% 12% | – – – | – 0 0 | – 0 0 |
| Right side SALT | 1.8% 0% 0% | 0% 0% 0% | 0% 0% 0% | 1.8% 0.9% 0.9% |
| Right side improvement I_{12}, I_{34} | – 100% 100% | – – – | – – – | – 50% 50% |
| Left side improvement I_{12}, I_{34} | – – – | – – – | – – – | – 0 0 |
| Left side SALT | 1.8% 0% 0% | 0% 0% 0% | 0% 0% 0% | 1.8% 0.9% 0.9% |
| Left side improvement I_{12}, I_{34} | – 100% 100% | – – – | – – – | – 50% 50% |
| Right side improvement I_{12}, I_{34} | – – – | – – – | – – – | – 0 0 |
| Posterior SALT | 22.8% 9.6% 4.8% | 18% 7.2% 4.8% | 4.8% 2.4% 1.2% | 8.4% 3.6% 2.4% |
| Posterior improvement I_{12}, I_{34} | – 57% 79% | – 60% 73% | – 50% 75% | – 57% 71% |
| Posterior improvement I_{24:12} | – – 50% | – – 33% | – – 50% | – – 33% |
| Total scalp SALT | 24.6% 17.6% 16.8% | 23.4% 7.2% 4.8% | 19.4% 12.2% 9% | 40% 22.5% 18.2% |
| Improvement in injection site I_{12}, I_{34} | – 60% 80% | – 69% 79% | – 37% 53% | – 43% 54% |
| Improvement in injection site I_{24:12} | – – 50% | – – 33% | – – 26% | – – 19% |
of endothelial cells, fibroblasts and skin cells. Studies also show that, in addition to proangiogenic, chemoattractive and anti-inflammatory potential, MSCs may modulate the activity of the immune system. Attempts have recently been made to use MSCs to reactivate hair follicle stem cells and thus prevent hair loss.

There are only a few reports in the medical literature on the use of MSCs in the treatment of alopecia. The most commonly used MSCs in AA therapy are those derived from bone marrow or adipose tissue. In a therapeutic experiment, autologous MSCs derived from bone marrow were used on a group of 40 people with hair loss, including 20 people with AA and 20 people with AGA. Six months after a single injection of stem cells into the scalp, the authors observed a significant improvement, confirmed with digital dermoscopy. There was no significant difference in the effectiveness of treatment between the 2 types of alopecia. No serious adverse events were reported.

Another interesting study reported the use of human autologous adipose-derived adult cells of stromal vascular fraction (ADSVC) for the treatment of 20 AA patients. Patients were given a single injection of autologous ADSVC cells extracted by lipoaspiration from adipose tissue into the alopecia foci at concentrations of $4 \times 10^6$ cells. The growth and thickness of the hair improved significantly within the first 6 months after treatment. A decrease in the intensity of the hair pull test was also observed. No side effects of ADSVC treatment were observed, and patients assessed the therapy as satisfactory.

Mesenchymal stem cells can also be extracted from WJ in the umbilical cord. It is an ideal source of stem cells due to its availability, noninvasive and painless extraction, weak immunogenic potential, no risk of adverse effects for the donor or recipient, and no ethical restrictions. Medical literature suggests the potential effectiveness of WJ-MSCs in hair follicle regeneration and hair regrowth. An additional advantage of using material extracted from WJ is the possibility of obtaining its decellularized fraction (DWJM), which is considered an excellent natural biocompatible 3D scaffold. The DWJM, as a 3D scaffold, can be used as a regenerative drug to promote stem cell adhesion, penetration, growth, and multiplication both in vitro and in vivo.

In our study, a single suspension of allogeneic WJ-MSCs was injected into the selected AA foci at a concentration of $5 \times 10^6$ cells, followed by a six-month observation of the treatment effects. All patients who participated in this experimental study had previously been treated with standard procedures, but without significant long-term clinical improvements. All patients showed a reduction in the area of baldness by an average of 67%. None of the patients experienced complete hair regrowth. Notably, we observed improved hair regrowth dynamics in all cases in the first 3 months after the procedure, with an increase of 52.2% on average compared to the following 3 months, when the increase was 32% on average.
The case of patient 1 (BR) is particularly interesting, because in the 12th week of observation, the subject was diagnosed with hair loss in the frontal area where no intradermal injection of WJ-MSC suspension was applied. No hair loss was detected in the remaining area of the scalp, while in the injected sites (i.e., the parietal area, right and left side of the scalp), hair regrowth did occur. This case indicates that the applied therapy is effective only at the site of cell administration itself.

There are very few reports of allogeneic therapies with WJ-MSCs in the medical literature to date. The authors of these papers stress the safety of this type of therapy, including the lack of tumorigenic potential.22,32,38

Our experiment also evaluated the safety of the procedures used. No side effects were observed during the procedure or after application of the WJ-MSC suspension in the allogeneic system.

**Limitations**

The limitation of the study was the small number of patients enrolled.

**Conclusions**

The presented report supports the effectiveness and safety of the applied therapy – intradermal injections of an allogeneic WJ-MSC suspension. To our knowledge, this is the first clinical study to describe the application of an allogeneic MSC transplant in patients with AA. The results of treatment were positive, with hair growth observed in all participants, and the therapy was found to be safe, with no side effects. The question remains as to how many cells should be given to the patient to achieve full hair regrowth and how often the treatments should be repeated to achieve 100% therapy efficacy with no relapse. We emphasize the need to conduct further studies with a randomized control group.

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**References**

1. Pratt CH, King LE Jr, Messenger AG, Christiano AM, Sundberg JP. Alopecia areata. Nat Rev Dis Primers. 2017;3:17011. doi:10.1038/nrdp.2017.11

2. Safavi K. Prevalence of alopecia areata in the First National Health and Nutrition Examination Survey. Arch Dermatol. 1992;128(5):702. doi:10.1001/archderm.1992.01680105136027

3. Abedini R, Hallaji Z, Lajevardi V, et al. Quality of life in mild and severe alopecia areata patients. Int J Womens Dermatol. 2017;4(2):91–94. doi:10.1016/j.jiwd.2017.07.001

4. Burgdorf WHC, Plewling G, Wolff HH, Landthaler M. Dermatologia. Vol. 1. Lublin, Poland: Czelej; 2017.

5. Strazzulli LC, Wang EHC, Avilia L, et al. Alopecia areata: An appraisal of new treatment approaches and overview of current therapies. J Am Acad Dermatol. 2018;78(1):15–24. doi:10.1016/j.jaad.2017.04.1142

6. Simakou T, Butcher JP, Reid S, Henriquez FL. Alopecia areata: A multifactorial autoimmune condition. J Autoimmun. 2019;98:74–85.

7. Gilhar A, Etzioni A, Paus R. Alopecia areata. N Engl J Med. 2012;366(16):1515–1525. doi:10.1056/NEJMra1103442

8. Guo H, Cheng Y, Shapiro J, McEwloe K. The role of lymphocytes in the development and treatment of alopecia areata. Expert Rev Clin Immunol. 2015;11(12):1335–1351. doi:10.1517/1744666X.2015.1085306

9. Li Y, Yan B, Wang H, et al. Hair regrowth in alopecia areata patients following Stem Cell Educator therapy. BMC Med. 2015;13:87. doi:10.1186/s12916-015-0331-6

10. Sudnik W. Rola selektyn E, L, P w patomechanizmie łysienia plackowatego [PhD thesis]. Poznan, Poland: Poznan University of Medical Sciences; 2012.

11. Hordinsky MK. Treatment of alopecia areata: What is new on the horizon? Dermatol Ther. 2011;24(3):364–368. doi:10.1111/j.1529-8019.2011.01421.x

12. Biran R, Zlotogorski A, Ramot Y. The genetics of alopecia areata: New approaches, new findings, new treatments. J Dermatol Sci. 2015;78(1):11–20. doi:10.1016/j.jdermsci.2015.01.004

13. Hordinsky MK. Overview of alopecia areata. J Invest Dermatol Symp Proc. 2013;16(1):513–515. doi:10.1038/jid.ysym.2013.4

14. Luczak M, Luczak T, Ciesińska C, Czajkowski R. Leczenie ogólne lisyenia plackowatego. Przegl Dermatol. 2013;100:53–58.

15. Nowicka D, Maj J, Jankowska-Konurs A, Hrynczewicz-Gwóźdź A. Efficacy of diphenylcyclopeneponine in alopecia areata: A comparison of two treatment regimens. Postepy Dermatol Allergol. 2018;35(6):577–581. doi:10.5114/ad.2018.77608

16. Dainichi T, Kabashima K. Alopecia areata: What’s new in epidemiology, pathogenesis, diagnosis, and therapeutic options? J Dermatol Sci. 2017;86(1):3–12. doi:10.1016/j.jdermsci.2016.10.004

17. Szabolowska-Gadowska I, Buzarska L, Malecki M. Właściwości komórek macierzystych, regulacje prawne oraz zastosowanie w medycynie. Postepy Hig Med Dosw (Online). 2017;71:1216–1230.

18. Marino L, Castaldi MA, Rosamilo R, et al. Mesenchymal stem cells from the Wharton’s jelly of the human umbilical cord: Biological properties and therapeutic potential. Int J Stem Cells. 2019;12(2):218–226. doi:10.15283/jiscs18034

19. Bajek A, Ołkowski J, Drewa T. Mezencymalne komórki macierzyste narzędzi terapeutycznych w regeneracji tkainek i narządów. Postepy Hig Med Dosw. 2011;65:124–132.

20. Wang HS, Hung SC, Peng ST, et al. Mesenchymal stem cells in the Wharton’s jelly of the human umbilical cord: Biological properties and therapeutic potential. Int J Stem Cells. 2019;12(2):218–226. doi:10.15283/jiscs18034

21. Lifshitz-Silver A, Landthaler M. Multipotent mesenchymal stromal cells: The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8(4):315–317. doi:10.1080/14653240600855905

22. Pojda Z, Machaj E, Kurzyk E, et al. Mezencymalne komórki macierzyste. Adv Biochem. 2013;39(2):187–197.

23. Martin PG, Martinez AR, Lara VG, Naveros BC. Regulatory considerations in production of a cell therapy medicinal product in Europe to clinical research. Clin Exp Med. 2014;14(1):23–33. doi:10.1007/s10238-012-0213-6

24. Masłowska L, Papirocka M, Czyżewska-Buczyńska A, et al. Autotransplantation of the adipose tissue-derived mesenchymal stem cells in therapy of venous stasis ulcers. Arch Immunol Ther Exp (Warsz). 2020;68(1):5. doi:10.1007/s00005-020-00571-9

25. Szydlak R. Produkty lecznicze zaawansowanej terapii medycznej oparte na mezenchymalnych komórkach. Postepy Dermatol Allergol. 2017;86(1):3–12.

26. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells: The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8(4):315–317. doi:10.1080/14653240600855905
28. Olsen EA, Hordinsky MK, Price VH, et al; National Alopecia Areata Foundation. Alopecia areata investigational assessment guidelines. Part II. National Alopecia Areata Foundation. J Am Acad Dermatol. 2004;51(3):440–447.

29. Maxson S, Lopez EA, Yoo D, Danilkovitch-Miagkova A, Leroux MA. Concise review: Role of mesenchymal stem cells in wound repair. Stem Cells Transl Med. 2012;1(2):142–149. doi:10.1002/stc.90

30. Lopez JF, Sarkanen JR, Huttala O, Kaartinen I, Kuokkanen HO, Ylikomi T. Adipose tissue extract shows potential for wound healing: In vitro proliferation and migration of cell types contributing to wound healing in the presence of adipose tissue preparation and platelet rich plasma. Cytotechnology. 2018;70(4):1193–1204. doi:10.1007/s10616-018-0211-y

31. Otero-Viñas M, Falanga V. Mesenchymal stem cells in chronic wounds: The spectrum from basic to advanced therapy. Adv Wound Care (New Rochelle). 2016;5(4):149–163. doi:10.1089/wound.2015.0627

32. Owczarczyk-Saczonek A, Krajewska-Włodarczyk M, Kruszewska, A, et al. Therapeutic potential of stem cells in follicle regeneration. Stem Cells Int. 2018;2018:1049641. doi:10.1155/2018/1049641

33. Egger A, Tomic-Canic M, Tosti A. Advances in stem cell-based therapy for hair loss. Cell Mol Repair Replace Regen Reprogram. 2020;8:e2894.

34. Anderi R, Makdissi N, Azar A, Rizk F, Hamade A. Cellular therapy with human autologous adipose-derived adult cells of stromal vascular fraction for alopecia areata. Stem Cell Res Ther. 2018;9(1):141. doi:10.1186/s13287-018-0889-y

35. Sabapathy B, Sundaram SVM, Mankuzhy P, Kumar S. Human Wharton’s jelly mesenchymal stem cells plasticity augments scar-free skin wound healing with hair growth. PLoS One. 2014;9(4):e93726. doi:10.1371/journal.pone.0093726

36. Ibrahim ZA, Elmaadawi IH, Mohamed BM, et al. Stem cell therapy as a novel therapeutic intervention for resistant cases of alopecia areata and androgenetic alopecia. J Dermatol Treat. 2018;29(5):431–440. doi:10.1080/09546634.2016.1227419

37. Jadalannagari S, Converse G, McFall C, et al. Decellularized Wharton’s jelly from human umbilical cord as a novel 3D scaffolding material for tissue engineering applications. PLoS One. 2017;12(2):e0172098. doi:10.1371/journal.pone.0172098

38. Gentile P, Garcovich S. Advances in regenerative stem cell therapy in androgenic alopecia and hair loss: Wnt pathway, growth-factor, and mesenchymal stem cell signaling impact analysis on cell growth and hair follicle development. Cells. 2019;8(5):466. doi:10.3390/cells8050466