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STEM CELLS TRANSLATIONAL MEDICINE

The use of umbilical cord-derived mesenchymal stem cells in patients with muscular dystrophies: Results from compassionate use in real-life settings

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Abstract

Muscular dystrophies are genetically determined progressive diseases with no cause-related treatment and limited supportive treatment. Although stem cells cannot resolve the underlying genetic conditions, their wide-ranging therapeutic properties may ameliorate the consequences of the involved mutations (oxidative stress, inflammation, mitochondrial dysfunction, necrosis). In this study, we administered advanced therapy medicinal product containing umbilical cord-derived mesenchymal stem cells (UC-MSCs) to 22 patients with muscular dystrophies. Patients received one to five intravenous and/or intrathecal injections per treatment course in up to two courses every 2 months. Four standard doses of 10, 20, 30, or 40 $\times$ 10^6 UC-MSCs per injection were used; the approximate dose per kilogram was 1 $\times$ 10^6 UC-MSCs. Muscle strength was measured with a set of CQ Dynamometer computerized force meters (CQ Elektronik System, Czernica, Poland). Statistical analysis of muscle strength in the whole group showed significant improvement in the right upper limb (+4.0 N); left hip straightening (+4.5 N) and adduction (+0.5 N); right hip straightening (+1.0 N), bending (+7.5 N), and adduction (+2.5 N); right knee straightening (+8.5 N); left shoulder revocation (+13.0 N), straightening (+5.5 N), and bending (+6.5 N); right shoulder adduction (+3.0 N), revocation (+10.5 N), and bending (+5 N); and right elbow straightening (+9.5 N); all these differences were statistically significant. In six patients (27.3%) these changes led to improvement in gait analysis or movement scale result. Only one patient experienced transient headache and lower back pain after the last administration. In conclusion, UC-MSC therapy may be considered as a therapeutic option for these patients.

KEYWORDS

dystrophy, muscular diseases, musculoskeletal disorders, stem cell therapy, Wharton's jelly

Lessons learned

The administration of Wharton's jelly-derived mesenchymal stem cells in patients with muscular dystrophies improves average muscle strength measured with a dynamometer and gait in some patients.

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Significance statement

Administration of Wharton's jelly-derived mesenchymal stem cells neurological indications is controversial. This study showed that cell therapy is a reasonable experimental treatment option, although the eligibility criteria for treatment need to be optimized.

1 | INTRODUCTION

Muscular dystrophies are genetic disorders that cause progressive wasting and weakness of skeletal muscle, leading to death by respiratory muscle weakness or cardiomyopathy, typically in the third decade of life.[1] Although over 30 unique genes are involved in the pathogenesis of muscular dystrophies, a similar mutation in the same gene may cause a wide range of phenotypes, and distinct genes may be responsible for one identical phenotype.[2,3] Because of this heterogeneity, pharmacologic treatments are limited. The current options include pharmacotherapy and supportive care, which involves physical and occupational therapy, orthopedic surgery, genetic counseling, mechanical ventilation, the use of implanted cardiac devices, and the management of comorbidities (such as cardiomyopathy, osteoporosis, and respiratory failure).[4] Steroids are the gold standard in pharmacotherapy, but they have significant side effects, including weight gain, short stature, puberty delay, behavioral issues, and pathologic bone fractures.[5] Tadalafil and sildenafil are two experimental drugs that have been used in the treatment of muscular dystrophies. Clinical studies in Becker muscular dystrophy[6] and Duchenne muscular dystrophy (DMD)[7] have reported encouraging results, but another trial testing sildenafil in DMD patients was prematurely terminated because of potential detrimental effects on heart function.[8] Ataluren and eteplirsen are intended for use only in 10%-15% of patients with strictly defined types of mutation. Among six studies assessing gene therapies, five (NCT04240314, NCT03375164, NCT03333590, NCT03362502, NCT03652259) involve 34 participants altogether. The biggest one (NCT04281485), involving 99 participants, will be completed in 2027. Because proper treatment is limited, there is an urgent need to look for other methods to stop the progress of the disease. Stem cell therapy is a promising option,[9,10] especially in DMD, also known as "muscle stem-cell disease."[11] This disease is characterized by dystrophin deficiency, leading to mitotic spindle orientation impairment and reduced myogenic progenitor cell generation.[11] In addition, dystrophin deficiency leads to increased permeability of sarcolemma, oxidative stress, inflammation, mitochondrial dysfunction, and finally necrosis with loss of muscle function.[12] Satellite cell dysfunction contributes also to the progressive muscle atrophy in myotonic dystrophy[13,14] and limb-girdle dystrophy.[15] In facioscapulohumeral muscular dystrophy, expression of double homeobox protein 4 (DUX4) is toxic to skeletal muscle and results in oxidative stress[16] and inflammatory muscle infiltration (typically with CD4 or CD8 cells),[17] which create a target for the immunoregulatory properties of mesenchymal stem cells; in this type of disease, impairment in satellite cells was also observed.[18,19]

A heterogeneous[20] population of adult stem cells, known as satellite cells, was described in 1961.[21] To date, several distinct types of myogenic progenitor cells have been described, among which two vessel-associated cell populations with myogenic ability seem to be crucial: pericytes and mesoangioblasts. After intravenous administration, these cells can cross the vessel wall and contribute to muscle regeneration.[22,23] However, direct transplantation of myogenic precursor cells is difficult. Unfortunately, the vast majority of myoblasts do not migrate into damaged muscle but die shortly after injection.[24] Even after local injection, migration is very limited.[25] Adult mesenchymal stem/stromal cells (MSCs) are primarily isolated from bone marrow (BM) but may also be isolated from placenta, umbilical cord (UC), amniotic fluid, adipose tissue, dental pulp, breast milk, and synovium.[26] They are currently being tested for several indications because of the very wide range of potential mechanisms of action.[26] In muscular dystrophies, MSCs may act as a therapeutic agent in several ways. First, they can differentiate into muscular progenitors and myocytes, which can fuse with damaged tissue and restore expression of dystrophin, as was shown in the mdx mouse model of DMD.[27] Second, they secrete immunomodulatory, anti-inflammatory, anti-apoptotic, neovascular, and proangiogenic factors, such as cytokines, chemokines, or growth factors, which improve milieu by decreasing inflammation, increasing oxygen supply, and ameliorating trophic conditions.[28,29] Third, they improve the neural component of the disease. Disruption of the dystrophin-glycan complex affects cerebral cortex layering and neuron function in patients with DMD and in DMD mouse models (mdxCv3 and dystroglycan knockout); reduced b-wave amplitudes in electroretinograms support the hypothesis about the role of the nervous system in pathogenesis.[30] Recent studies also showed neural impairment in other types of dystrophies.[31,32]

MSCs obtained from umbilical cord have many advantages over MSCs isolated from another sources. A comprehensive literature review by Mattar et al demonstrated superiority of Wharton's jelly (WJ)-derived MSCs (WJ-MSCs) over BM-derived MSCs (BM-MSCs) in immunogenicity, proliferation and senescence and equivalence or superiority in immunomodulatory properties.[33] In 2017, WJ-MSCs were shown in vitro to secrete 55 times more hepatocyte growth factor (HGF) (which stimulates myogenesis, cell migration, and immunoregulation) than BM-MSCs, and these observations were confirmed by efficacy in a randomized phase I/II clinical trial: only WJ-MSC-treated patients had increased left ventricular ejection fraction 3, 6,

and 12 months after cell administration in patients with heart failure.[34] Also, in terms of neuroprotective properties, WJ-MSCs were superior to BM-MSCs: they secreted more of eight of nine evaluated growth factors with angiogenic or neuroprotective effects (ninefold more in the case of HGF), which translated into 20% more surviving stressor-treated nerve cells, although both BM-MSCs and WJ-MSCs resulted in significant neurite growth.[35] Therefore, some opine that WJ-MSCs should be the new gold standard in cell therapy.[36-39] Their regenerative properties in muscular disorder was shown in a zebrafish model.[40]

According to Polish and European law, WJ-MSCs may also be used within the legal frame of the medical therapeutic experiment, concerning the compassionate use of a medicinal product, even for indications for which they are not registered. The aim of this paper was to describe the results of the compassionate use of WJ-MSCs in patients with muscular dystrophies treated in real-life settings.

2 | MATERIALS AND METHODS

This medical experiment was conducted in the Klara Medical Center in Częstochowa, Poland, between October 2016 and February 2018. The experiment was designed as a single arm open label study. Patients were recruited from the daily practice of neurologists working at this hospital. Inclusion criteria were as follows: women and men aged 3-85 years and diagnosis of dystrophy, regardless of type. Women of childbearing age had to use contraception. Exclusion criteria included the following: pregnancy or breastfeeding, infectious disease, severe psychiatric disorders, carcinoma, penicillin intolerance, and unstable cardiovascular disease. Ethical approval was obtained from the local Bioethical Committee in Częstochowa (K.B.Cz.-0003/2016 on June 15, 2016; K.B.Cz.-0005/2016 on September 21, 2016; K.B.Cz.-0007/2016 on November 9, 2016; and K.B.Cz.-0003/2017 on February 15, 2017). Although the experiment was not registered as a clinical study (the nature and legal position of the medical experiment outside clinical trials in the Polish legal system has been previously described[41]), it was conducted according to the Declaration of Helsinki. All patients signed an informed consent form before the first administration.

2.1 | Product harvesting and processing

The medicinal product used in this experiment was manufactured by Polski Bank Komórek Macierzystych S.A. (FamiCord Group, Warsaw, Poland) in accordance with Good Manufacturing Practice under the supervision of Chief Pharmaceutical Inspectorate, Warsaw, Poland. The manufacturing process was described earlier in detail.[42] In brief, umbilical cords were donated by healthy Polish newborns after maternal qualification based on a medical questionnaire conducted after the parents signed an informed consent form. The harvested UCs were transported to the laboratory under monitored conditions and processed within 48 hours of delivery. After disinfection, they were dissected, stripped of any blood vessels, and minced into 2-cm^3 pieces, which were placed into six-well plates. The tissue explants were cultured at 37°C in 5% CO_2 in air. After 1-2 hours, the non-adherent cells were washed off, and the attached cells were further expanded. The tissue explants were removed after 2-3 weeks of culture. When the adherent cells reached 90% confluence, they were passaged and reseeded for further expansion at 1.2×10^4 cells per cm^2 in a 75-cm^2 tissue culture flask (Becton, Dickinson and Company, Franklin Lakes, NJ). To evaluate their numbers, the cells were detached and counted in a hemocytometer. When a sufficient number of cells was reached, they were immunophenotyped, cryopreserved, and stored in the vapor phase of liquid nitrogen. The cells fulfilled the criteria described by Dominici et al.[43] The characteristics of the product are presented in Table 1.

TABLE 1 Characteristics of the cells administered to the patients

Characteristic	Value/specification
Viability	>90%
Morphology	Surface-adherent, fibroblast-like
Positive markers	CD73, CD90, CD105
Negative markers	CD34, CD45, CD14, CD19, HLA-DR
Passage	≤5th
Infectious agent tests	HIV RNA, HCV RNA, HBV DNA, anti-HIV I/II, anti-HCV, anti-HBc, HBs-Ag, Anti-CMV IgM, anti-CMV IgG, anti-Toxo IgM, anti-Toxo IgG, syphilis
Other quality tests	Evaluation for post-thawed sterility, prior to cryopreservation and post-thawed cell number and cell viability, post-thawed immunophenotype as well as functional test (ability for ex vivo proliferation)
Cryoprotectant	10% dimethylsulfoxide solution in 5% human serum albumin

Abbreviations: CMV, cytomegalovirus; HBc, hepatitis B core antibody; HBs-Ag, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HLA-DR, human leukocyte antigen-DR isotype; Toxo, toxoplasmosis.

2.2 | Treatment administration

Treatment consisted of one to five intravenous injections per one treatment course of advanced therapy medicinal product containing WJ-MSCs. Up to two treatment courses were permitted. Four standard doses of 10, 20, 30, or 40×10^6 MSCs per injection were used. The total stem cell count varied according to the weight of the patient, but the approximate dose per kilogram was 1×10^6 MSCs. Injections were administered every 2 months after patient qualification by the study investigator. The dose, scheme, and route of administration was selected on the basis of papers reporting positive effects of WJ-MSC administration in another neurological diseases.[41,44] These experiments were conducted by other teams using the same medicinal product delivered by the same manufacturer. Previous

papers describing results obtained in other neurological indications revealed a dose-dependent therapeutic effect with the minimal effective dose corresponding to at least three administrations.[41,44] The first administration was always intravenous for safety reasons; the following injections were administered intravenously or intrathecally. The rational basis of intrathecal administration is the presence of dystrophin in myelin-forming Schwann cells[45] and additive effect of co-transplantation of myoblasts and Schwann cells.[46] This route of administration in muscular dystrophy was tested by Sharma et al in 2013 who described subjective and functional improvement in 97% of 38 treated patients as well as amelioration in magnetic resonance imaging or electromyography in five patients.[47]

The patients did not receive immunosuppressive treatment. MSCs express major histocompatibility complex class II molecules at very low levels and do not express the co-stimulatory molecules CD40, CD40 ligand, CD80, and CD86.[48] Although some authors described immunogenicity after administration of BM-MSCs, this possibility was observed only in BM-MSCs and was ruled out for UC-derived MSCs (UC-MSCs).[49] A systematic review of clinical trials using WJ-MSCs did not report even a single case of rejection of these cells in a group of more than 2000 patients.[50] Therefore, immunosuppressive treatment was considered not necessary.

2.3 | Clinical assessment

Clinical assessments (including gait kinetics, Brooke scale, and Vignos scale—only in patients who were able to walk) were carried out during each hospitalization, at least 1 hour after the afternoon meal on the day before the administration of MSCs. The assessments took place in two rooms: the first with a centipede grid on the floor and wall for gait kinetics tests and the second with the Universal Cabinet of Medicinal Improvement for muscle strength tests. Patients were dressed in a loose-fitting outfit that did not restrict movement (short-sleeved shirt and tracksuit trousers).

2.4 | Tests investigating gait kinetics

Patients were tested twice. In the first test, they were placed in front of a camera recording the gait, against the background of the centipede grid. The patient walked 2 m toward the camera, made a rotation, and returned the same way to the wall. From the recording, the researcher measured sideways leanings in the frontal plane. In the second test, the patient walked along the wall for 6 m, oriented sideways to the camera (corridor test). The results considered the length of the step and the time it took to cover this distance.

2.5 | Tests investigating muscle strength

The tests were performed with a set of CQ Dynamometer computerized force meters (CQ Elektronik System, Czernica, Poland). All patients were asked to perform 26 muscle strength tests, with three iterations for each test. The first two tests were performed with a dynamometer and concerned the bending force of the fingers of the upper limbs. The next 24 tests concerned the muscle strength of both upper and lower limbs, with six tests per limb. The bending and straightening force in shoulder, elbow, and hip and knee joints, as well as the adduction and revocation force in shoulder and hip joints, were tested. Once the test subject was stabilized, the test joint was positioned at right angles and the trunk perpendicular to the ground. A dynamometer was then placed, the force arm was measured, and the force measurement was taken. The trunk flexors and extensors were measured with the pelvis stabilized. During the measurement of the knee joint, the subject sat on a bench with his or her hands on the edge of the bench top. The tested limb was suspended by a system of suspensions and blocks so that muscle strength was transferred to a strain gauge (stress sensor) by means of a cord. The results were converted to a force moment (force $\times$ arm length [Nm]). The highest result (force moment), given in Newtons [N], was considered.

2.6 | Statistical analysis

Results (comparison between baseline and end of therapy) were processed with descriptive statistics and reported as medians and interquartile ranges. Differences between baseline and the following administrations were compared using the Friedman analysis of variance followed by Dunn's multiple comparison test (post hoc). Values of $P < .05$ were considered significant. Trend in 6-month follow-up was described qualitatively.

3 | RESULTS

The study group included 22 patients: 11 men and 11 women. The median age was 33 years, range 4 to 63 years, and interquartile range 30 to 38 years. Among the patients, 10 had limb-girdle muscular dystrophy, six had facioscapulohumeral muscular dystrophy, one had myotonic dystrophy, one had Becker dystrophy, and four had an unspecified form of this disease. In two cases, the route of administration was always intravenous; in four it was always intrathecal. Sixteen patients received one intravenous injection followed by intrathecal injections on other visits.

Overall, the individual response to treatment was heterogenous. In the most successful case, the patient began moving without a crutch, stopped rehabilitation, and rejoined a full-time job. Gait analysis was assessed qualitatively; the investigator's notes are presented in Table 2. Statistical analysis of muscle strength [N] in the whole group showed significant improvement in the right upper limb (175.5 [99.5-293.2] vs 179.5 [118.2-198.7], $P = .049$); left hip straightening (21.5 [11.0-36.2] vs 26.0 [11.0-51.2], $P = .014$) and adduction (8.0 [3.0-35.7] vs 8.5 [3.7-57.5], $P = .018$); right hip straightening (22.0 [10.5-41.0] vs 23.0 [11.0-63.5], $P = .046$), bending (18.0 [5.0-37.0] vs 25.5 [8.0-55.2], $P = .003$), and adduction (7.5 [2.7-44.7] vs 10.0

TABLE 2 Gait assessment and functional assessment

Type	Age	Sex	No. of injections		General assessment (interview and observation)				Muscle strength (measured)	Gait analysis			Tendency in follow-up after 6 months[a]	Brooke scale		Vignos scale		Adverse events
			i.v.	i.t.	Investigator's opinion	Patient's opinion	Walking	Self-service		Frontal inclinations	Step length	Walking speed		Baseline	End of therapy	Baseline	End of therapy	
Becker	20	M	5	1	0	0	0	0	=	0	0	0	=	2	2	2	2	No
Becker	24	M	1	4	+	+	+	+	+	0	0	0	=	1	1	2	2	No
Limb-girdle	27	F	4	4	0	0	+	0	=	0	0	0	↓	3	3	5	5	No
Limb-girdle	31	F	1	4	0	0	0	0	=	0	0	0	=	4	4	3	3	Weight gain
Limb-girdle	32	F	7	0	0	+	0	+	+	0	0	0	↑	4	4	7	7	No
Limb-girdle	36	F	2	3	+	−	0	0	+	0	0	0	↑	2	2	2	2	No
Limb-girdle	38	M	1	4	0	0	0	0	=	0	0	0	=	5	5	9	9	Headache, lower back pain
Limb-girdle	39	F	1	4	+	+	+	+	+	0	0	0	↑	4	3	7	5	No
Limb-girdle	45	M	1	4	0	0	0	0	=	0	0	0	↓	3	3	8	8	No
Limb-girdle	63	F	1	8	+	+	0	0	+	0	0	0	=	4	3	7	7	No
Myotonic	4	M	8	1	+	+	+	0	+	+	+	+	↑	1	1	3	3	No
Myotonic	38	M	1	7	+	+	+	0	=	0	0	0	=	3	3	3	3	No
Duchenne	29	F	1	4	+	0	+	0	=	+	+	+	=	2	2	4	4	No
FSHD	27	M	3	2	0	+	+	+	=	0	0	0	=	4	4	7	7	No
FSHD	32	M	1	9	0	+	+	+	+	+	+	+	=	2	2	1	2	No
FSHD	32	F	4	4	+	+	+	+	+	0	0	0	=	2	1	2	3	No
FSHD	33	M	3	3	0	0	0	0	=	0	0	0	=	3	3	5	5	No
FSHD	34	F	3	3	0	+	+	+	+	0	0	0	↑	3	3	5	5	No
FSHD	42	F	6	3	+	+	+	0	+	0	0	0	=	3	3	7	7	No
Not specified	33	M	1	6	+	+	+	+	+	0	0	0	↑	2	2	2	3	No
Not specified	35	F	1	4	+	+	0	+	+	0	0	0	=	2	2	3	3	No
Not specified	45	M	1	9	0	+	+	+	=	0	0	0	↑	2	2	9	9	No
Improved, n (%)					11 (50.0)	14 (63.6)	13 (59.1)	10 (45.5)	12 (54.5)	3 (13.6)	3 (13.6)	3 (13.6)	7 (31.8)	3 (13.6)		1 (4.5)		

[a]Qualitative.

Abbreviations: F, female; FSHD, facioscapulohumeral muscular dystrophy; i.t., intrathecal; M, male.

TABLE 3 Change in muscle strength over time (n = 22)

Assessed function and location Injection Assessment	Visit 1 First Baseline	Visit 2 Second After first	Visit 3 Third After second	Visit 4 Fourth After third	Visit 5 Fifth After fourth	*P* value
Right upper limb						
Right upper limb	175.5 (99.5-293.2)	165.5 (100.2-273.2)	175.0 (107.5-275.0)	183.0 (108.7-270.0)	179.5 (118.2-198.7)	NS (.061)
Baseline vs EOC	175.5 (99.5-293.2)	—	—	—	179.5 (118.2-198.7)	.049
% vs baseline	—	−2.2 (−9.1; 19.1)	3.3 (−7.1; 16.6)	6.6 (−3.8; 22.5)	11.8 (−0.5; 24.7)	NS
Left upper limb						
Left upper limb	188.0 (101.0-269.7)	202.0 (96.5-279.7)	184.0 (102.2-299.5)	177.0 (122.0-275.7)	191.5 (124.0-298.0)	NS (.404)
Baseline vs EOC	188.0 (101.0-269.7)	—	—	—	191.5 (124.0-298.0)	NS (.114)
% vs baseline	—	−0.7 (−6.9; 7.3)	4.6 (−5.8; 8.0)	1.0 (−5.9; 12.0)	7.1 (−3.3; 16.1)	NS
Left hip						
Straightening	21.5 (11.0-36.2)	23.0 (12.2-47.2)	28.5 (9.5-41.2)	26.5[a] (12.2-61.2)	26.0 (11.0-51.2)	.010
Baseline vs EOC	21.5 (11.0-36.2)	—	—	—	26.0 (11.0-51.2)	.014
% vs baseline	—	7.7 (−16.4; 43.1)	9.8 (−15.8; 32.4)	20.2 (5.8; 50.7)	24.8 (−9.3; 37.6)	NS
Bending	17.5 (5.7-40.7)	16.0 (6.0-41.7)	13.0 (6.5-61.7)	14.0 (7.5-62.5)	17.0 (7.5-72.2)	NS (.104)
Baseline vs EOC	17.5 (5.7-40.7)	—	—	—	17.0 (7.5-72.2)	NS (.094)
% vs baseline	—	1.2 (−27.1; 29.1)	3.8 (−20.4; 26.2)	23.1 (−18.7; 91.1)	19.6 (−9.5; 61.7)	NS
Adduction	8.0 (3.0-35.7)	8.5 (3.0-51.2)	7.5 (2.7-58.7)	7.0 (2.7-55.7)	8.5 (3.7-57.5)	NS (.068)
Baseline vs EOC	8.0 (3.0-35.7)	—	—	—	8.5 (3.7-57.5)	.018
% vs baseline	—	18.2 (0; 50.0)	2.4 (−20.0; 52.8)	9.8 (−20.0; 51.9)	25.2 (−1.2; 79.5)	NS
Revocation	23.0 (13.0-35.2)	25.0 (14.0-46.5)	24.5 (15.0-64.7)	26.5[a] (14.7-57.7)	24.5 (14.0-59.2)	.013
Baseline vs EOC	23.0 (13.0-35.2)	—	—	—	24.5 (14.0-59.2)	NS (.090)
% vs baseline	—	3.8 (−6.7; 22.4)	11.7 (−4.6; 35.0)	15.3 (5.2; 34.2)	10.0 (−11.3; 26.2)	NS
Right hip						
Straightening	22.0 (10.5-41.0)	23.0 (8.7-44.2)	16.5 (8.5-49.7)	21.0 (10.0-61.7)	23.0 (11.0-63.5)	.018
Baseline vs EOC	22.0 (10.5-41.0)	—	—	—	23.0 (11.0-63.5)	.046
% vs baseline	—	0 (−18.1; 20.8)	−6.5 (−22.9; 32.9)	7.6 (−1.0; 43.5)	8.7 (−6.0; 61.2)	NS
Bending	18.0 (5.0-37.0)	23.0 (7.7-38.0)	17.5 (6.0-43.0)	20.0 (5.7-50.2)	25.5[b] (8.0-55.2)	.003
Baseline vs EOC	18.0 (5.0-37.0)	—	—	—	25.5 (8.0-55.2)	.002
% vs baseline	—	2.9 (−14.3; 62.5)	20.0 (−4.1; 56.5)	27.5 (−5.2; 66.7)	42.9 (6.5; 100.0)	NS
Adduction	7.5 (2.7-44.7)	8.5 (3.7-54.0)	10.0[a] (3.0-79.5)	10.5[a] (4.5-84.7)	10.0[c] (4.7-80.2)	<.0001
Baseline vs EOC	7.5 (2.7-44.7)	—	—	—	10.0 (4.7-80.2)	<.0001
% vs baseline	—	25.1 (−2.5; 100.0)	35.3 (0; 55.7)	25.8 (0; 150.5)	46.3 (19.1; 121.1)	NS

TABLE 3 (Continued)

Assessed function and location Injection Assessment	Visit 1 First Baseline	Visit 2 Second After first	Visit 3 Third After second	Visit 4 Fourth After third	Visit 5 Fifth After fourth	*P* value
Revocation	20.5 (10.2-42.7)	21.5 (11.2-44.5)	22.5 (9.0-51.2)	22.0 (9.7-58.7)	19.5 (10.2-63.0)	NS (.297)
Baseline vs EOC	20.5 (10.2-42.7)	—	—	—	19.5 (10.2-63.0)	NS (.085)
% vs baseline	—	8.3 (−15.0; 39.3)	−9.4 (−18.6; 31.0)	13.3 (−4.7; 33.3)	5.2 (−15.9; 43.1)	NS
Left knee						
Straightening	38.0 (17.5-85.7)	48.5 (18.5-114.7)	41.5 (16.0-115.2)	43.5 (16.7-149.0)	41.0 (16.7-173.7)	NS (.671)
Baseline vs EOC	38.0 (17.5-85.7)	—	—	—	41.0 (16.7-173.7)	NS (.355)
% vs baseline	—	12.9 (−6.9; 65.0)	5.8 (−12.4; 61.8)	0 (−25.7; 60.0)	−3.6 (−17.9; 77.5)	NS
Bending	20.0 (13.5-51.2)	21.0 (9.5-65.2)	24.0 (13.7-77.7)	21.0 (9.2-76.2)	21.0 (8.5-81.7)	NS (.572)
Baseline vs EOC	20.0 (13.5-51.2)	—	—	—	21.0 (8.5-81.7)	NS (.935)
% vs baseline	—	6.7 (−19.5; 40.2)	9.4 (−8.1; 75.2)	0 (−26.5; 31.2)	−4.5 (−43.1; 21.9)	NS
Right knee						
Straightening	31.5 (13.7-76.0)	44.0 (15.0-107.0)	39.5 (13.2-96.7)	40.5 (16.0-97.5)	40.0 (16.7-111.5)	NS (.093)
Baseline vs EOC	31.5 (13.7-76.0)	—	—	—	40.0 (16.7-111.5)	.033
% vs baseline	—	6.7 (−19.5; 40.2)	9.4 (−8.1; 75.2)	0 (−26.5; 31.2)	−4.5 (−43.1; 21.9)	NS
Bending	21.0 (11.5-56.2)	18.0 (11.5-83.0)	22.0 (12.0-96.7)	26.0 (8.5-105.5)	29.0 (11.7-80.7)	NS (.517)
Baseline vs EOC	21.0 (11.5-56.2)	—	—	—	29.0 (11.7-80.7)	NS (.562)
% vs baseline	—	−2.6 (−36.8; 43.3)	−6.4 (−20.4; 68.6)	−9.8 (−31.1; 76.3)	0 (−25.4; 83.2)	NS
Left shoulder						
Adduction	6.5 (2.7-24.0)	7.5 (3.0-28.0)	7.0 (4.0-30.5)	9.0 (4.0-29.5)	9.0 (4.5-27.0)	NS (.553)
Baseline vs EOC	6.5 (2.7-24.0)	—	—	—	9.0 (4.5-27.0)	NS (.165)
% vs baseline	—	3.3 (−20.8; 31.1)	0 (−20.8; 49.2)	12.7 (−23.9; 102.5)	24.5 (−25.4; 57.9)	NS
Revocation	26.5 (11.0-46.0)	30.5 (13.5-59.0)	27.0 (18.2-59.0)	30.5[a] (17.2-62.0)	39.5[a] (14.5-66.0)	.005
Baseline vs EOC	26.5 (11.0-46.0)	—	—	—	39.5 (14.5-66.0)	.003
% vs baseline	—	14.6 (−0.7; 40.7)	7.9 (−12.4; 39.9)	19.7 (6.7; 52.3)	19.6 (−5.4; 85.8)	NS
Straightening	8.0 (3.7-34.0)	8.5 (3.0-39.2)	8.0 (3.7-30.7)	11.0[b] (4.7-36.2)	13.5[a] (4.0-34.7)	.001
Baseline vs EOC	8.0 (3.7-34.0)	—	—	—	13.5 (4.0-34.7)	.017
% vs baseline	—	13.3 (−5.8; 35.0)	18.9 (−13.7; 75.2)	45.8 (12.4; 85.0)	20.1 (0; 125.0)	NS
Bending	14.0 (8.7-30.7)	18.0 (9.7-32.0)	20.0 (11.0-38.7)	20.0 (13.2-40.5)	20.5[c,d] (15.7-43.5)	<.0001
Baseline vs EOC	14.0 (8.7-30.7)	—	—	—	20.5 (15.7-43.5)	.001
% vs baseline	—	4.5 (−12.5; 34.9)	18.8 (−12.2; 47.1)	22.0 (0; 63.5)	49.6 (10.6; 67.5)	NS

(Continues)

TABLE 3 (Continued)

Assessed function and location Injection Assessment	Visit 1 First Baseline	Visit 2 Second After first	Visit 3 Third After second	Visit 4 Fourth After third	Visit 5 Fifth After fourth	*P* value
Right shoulder						
Adduction	7.0 (3.0-17.7)	6.5 (3.7-27.2)	8.0[e] (4.0-29.5)	8.0[b] (5.0-29.0)	10.0[c,d] (6.0-30.0)	<.0001
Baseline vs EOC	7.0 (3.0-17.7)	—	—	—	10.0 (6.0-30.0)	<.0001
% vs baseline	—	3.3 (−5.0; 48.8)	15.0 (−14.2; 58.9)	36.7 (6.9; 110.7)	33.3 (15.1; 115.4)	<.05
Revocation	23.0 (9.7-38.2)	27.0 (11.7-49.7)	30.5 (12.0-53.2)	34.0 (11.7-52.5)	33.5 (11.0-58.7)	NS (.159)
Baseline vs EOC	23.0 (9.7-38.2)	—	—	—	33.5 (11.0-58.7)	.021
% vs baseline	—	8.5 (−5.3; 50.0)	5.8 (−11.5; 56.5)	4.1 (−2.2; 55.8)	10.0 (−4.6; 45.0)	NS
Straightening	6.5 (4.0-26.5)	5.5 (3.0-28.0)	7.0 (4.5-29.7)	8.0[e] (4.0-32.7)	9.5 (2.7-35.7)	.008
Baseline vs EOC	6.5 (4.0-26.5)	—	—	—	9.5 (2.7-35.7)	NS (.064)
% vs baseline	—	0 (−35.0; 50.0)	24.2 (−22.1; 57.2)	14.3 (0; 100.0)	19.5 (−19.8; 93.6)	NS
Bending	16.0 (11.0-27.2)	17.0 (7.7-36.7)	18.0 (10.0-47.0)	22.5[a,d] (11.7-45.0)	21.0 (10.7-45.7)	.002
Baseline vs EOC	16.0 (11.0-27.2)	—	—	—	21.0 (10.7-45.7)	.020
% vs baseline	—	4.2 (−25.3; 56.2)	10.6 (−11.7; 44.1)	23.6 (0; 66.5)	7.7 (−8.7; 64.8)	NS
Right elbow						
Straightening	16.0 (9.7-52.5)	15.0 (11.0-72.2)	21.0[a] (12.2-75.7)	25.5[c,e] (14.5-85.7)	25.5[c] (14.7-85.5)	<.0001
Baseline vs EOC	16.0 (9.7-52.5)	—	—	—	25.5 (14.7-85.5)	<.0001
% vs baseline	—	24.1 (−1.7; 40.0)	32.2 (2.1; 81.1)	48.5 (24.9; 153.5)	59.4 (12.1; 207.0)	<.05
Bending	29.0 (10.5-46.0)	18.5 (14.5-50.0)	20.0 (15.2-74.5)	28.0 (15.5-124.2)	24.0 (13.7-187.2)	NS (.195)
Baseline vs EOC	29.0 (10.5-46.0)	—	—	—	24.0 (13.7-187.2)	.038
% vs baseline	—	14.2 (−28.6; 56.3)	33.3 (−29.3; 68.2)	16.6 (−23.3; 131.6)	39.3 (−22.2; 102.7)	NS

Notes: Results are given as median and interquartile range (25%-75%).
Abbreviations: EOC, end of the course; NS, not significant.
[a]$P < .05$ vs baseline.
[b]$P < .01$ vs baseline.
[c]$P < .001$ vs baseline.
[d]$P < .01$ vs first injection.
[e]$P < .05$ vs first injection.

[4.7-80.2], $P < .0001$); right knee straightening (31.5 [13.7-76.0] vs 40.0 [16.7-111.5], $P = .033$); left shoulder revocation (26.5 [11.0-46.0] vs 39.5 [14.5-66.0], $P = .003$), straightening (8.0 [3.7-34.0] vs 13.5 [4.0-34.7], $P = .017$), and bending (14.0 [8.7-30.7] vs 20.5 [15.7-43.5], $P = .001$); right shoulder adduction (7.0 [3.0-17.7] vs 10.0 (6.0-30.0), $P < .0001$), revocation (23.0 [9.7-38.2] vs 33.5 [11.0-58.7], $P = .021$), and bending (16.0 [11.0-27.2] vs 21.0 [10.7-45.7], $P = .020$); and right elbow straightening (16.0 [9.7-52.5] vs 25.5 [14.7-85.5], $P < .0001$). Full data are shown in Table 3.

In general, patients tolerated administrations well. Only one patient experienced transient headache and lower back pain after the last administration.

4 | DISCUSSION

It is widely known that efficient muscle regeneration depends on the presence of muscle satellite cells and muscle-resident mesenchymal progenitor cells, but much fewer studies describe the potentially therapeutic role of MSCs in muscle regeneration. Only a few papers describe the efficacy of these cells and the suggested mechanisms through which they may ameliorate the clinical symptoms of progressive muscle degeneration, other than the administration of genetically modified cells.[51,52] In 2017, Maeda et al reported that BM-MSCs transplanted into the peritoneal cavity of DMD mouse model specimens with severe muscle degeneration improved symptoms and extended survival from 9 to 29 weeks ($P < .01$).[53] Mice treated with BM-MSCs showed a significantly improved body size, gait, locomotor activity, and histopathological features of isolated single muscle fibers (intermediate number of branches and satellite cells between wild type and mdx mutant; inhibited fibrosis). Results from in vitro culture confirmed that isolated fibers increased their diameter because of increased Paired Box 7 (PAX7) under the influence of chemokine C-X-C Motif Chemokine Ligand 12 (CXCL12) secreted by BM-MSCs. The therapeutic properties of WJ-MSCs have also been observed in mice. In 2010, Vieira et al confirmed that these MSCs are able to reach and engraft in muscles after intravenous administration in a mouse model of limb-girdle muscular dystrophy. Disease progression in injected mice was slower in two of three blinded functional tests (inclined plane test and wire hanging test), despite lack of human dystrophin expression.[54] This suggests that these cells do not generate myofibers but enhance the differentiation rate of primary myogenic progenitors. Adipose tissue-derived MSCs seem to have similar properties. Microfragmented adipose tissue injected in DMD mouse model (mdx-bGeo) specimens improved the muscular phenotype of the mice, decreasing necrosis, fibrosis, and local proinflammatory cytokines. It also increased fiber size from 103 ± 22 μm^2 in the control group to 136 ± 24 μm^2 in the study group.[55] Improvement in model mice was also noticed after administration of placenta-derived MSCs.[56]

The genetic component of muscular dystrophies cannot be resolved, but the consequences of the involved mutation may be ameliorated. A potential mode of action may be related to the anti-inflammatory properties of these cells[57-60] because inflammatory processes are involved in the pathogenesis of muscular dystrophies.[61,62] For instance, the tumor necrosis factor-α serum concentration in patients with DMD was increased eight times compared with healthy boys of the same age,[63] and interleukin-6 was increased twice.[64] De Pasquale et al demonstrated an overexpression of interleukin-17 in muscle biopsies from patients with DMD and the negative correlation between this cytokine level and functional lower motor outcome.[65] Nonsteroidal anti-inflammatory drugs ameliorated muscle morphology, reduced macrophage infiltration and necrosis, and improved tolerance to fatigue.[66] Another mode of action may be the secretion of growth factors, especially platelet-derived growth factor, which increases the population of satellite cells and the number of regenerative fibers.[67]

Our study showed objective and significant improvement in muscle strength in 12 patients (54.5%). This amelioration resulted in improved gait in three patients and improved results in a motor scale in another three patients; altogether, in 6 of 22 (27.3%) patients the benefit from the therapy was significant enough to ameliorate their clinical parameters. Only one patient experienced partial deterioration (in lower limbs) during therapy and follow-up. Clinical outcome was not associated with type of dystrophy, sex, or route of administration. Our results agree with those obtained by other authors. In 2015, Li et al reported an improvement in the gait and muscle strength of three patients with Becker muscular dystrophy.[68] Also in 2015, Rajput et al described the administration of WJ-MSCs in 11 children with DMD and reported stabilization compared with a small ($n = 5$) control group.[69] In 2018, Dai et al described an improvement in electromyography in nine patients with DMD after intra-arterial administration of UC-MSCs.[70] Large randomized clinical studies should be performed to confirm these early observations. Besides basic muscle assessment, a clinical study might include an endpoint related to the lowered cognitive function observed in myopathies, including DMD,[71,72] and improved after WJ-MSC administration.[41,44] Currently, only one clinical trial registered on clinicaltrials.gov is evaluating BM-MSCs in patients with DMD (NCT03067831); the results of this study are expected in 2022.

It is far too early to determine the position of MSCs in the treatment of dystrophies. It is not known how long the therapeutic effect will last in extended follow-up, as no such studies have yet been conducted in dystrophic patients. Because of the presumably paracrine mechanism of action, it may be that the therapy should be repeated cyclically. Further studies are needed to optimize stem cell therapy in terms of both long-term treatment scheme and possible synergy with pharmacological drugs and/or rehabilitation.

5 | CONCLUSION

The administration of WJ-MSCs in muscular dystrophies is a reasonable experimental treatment available in Poland in a hospital exemption procedure. The results are cautiously encouraging, especially because there is no efficient registered treatment. However, we must emphasize limitations of this study related to (a) the open and

uncontrolled design of the analysis; (b) lack of measurement of secretion of biologically active compounds by cells contained in the injected medicinal product; (c) lack of blood test biomarkers for monitoring disease progression and safety as well as common clinical tests such as the 6-minute walk test, time to stand up, or the North Star Ambulatory Assessment; and (d) short and qualitative follow-up. Further experiments are required to confirm these findings and to identify the predictors for a positive clinical response.

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CONFLICT OF INTEREST

I.Z.-M. and D.B. are employees of Polski Bank Komórek Macierzystych S.A. (FamiCord Group), Warsaw, Poland. B.Ś.F. and D.S. declared leadership position with Klara Medical Center.

AUTHOR CONTRIBUTIONS

B.Ś.F.: conception/design, provision of study material or patients, collection and/or assembly of data, final approval of manuscript; I.Z.-M.: data analysis and interpretation, manuscript writing, final approval of manuscript; D.S.: administrative support, final approval of manuscript; D.B.: financial support, final approval of manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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