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Research Article

Safety and Efficacy of Bone Marrow Mesenchymal Stem Cell Extracellular Vesicles in Long COVID Patients: A Case Series

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Abstract

Long COVID, or Post Acute Sequelae of COVID-19 (PASC), is a prolonged, debilitating syndrome that follows acute SARS-CoV-2 infection in >10% of cases. With immunomodulatory and regenerative properties, human bone marrow mesenchymal stem cell derived extracellular vesicles (hBM-MSC EVs) may present a new therapeutic option. To explore this treatment option, we performed a prospective IRB safety study with an advanced BM-MSC EV investigational product (IP) and measured subject status using patient-reported outcome measures (PROMs). Ten subjects with confirmed long COVID symptoms received two intravenous 15 mL doses of IP one week apart. Safety events and subject status were monitored over six months. No serious adverse events occurred. Statistically significant improvements, as compared to baseline, were observed for the following PROMs as early as three weeks after first infusion and were sustained for six months: PROMIS (mental, physical, average pain), EQ-5D-5L, IES-R, PCFS, and FSS. No improvement was detected by SF-36. Nor was improvement indicated by the cognitive assessments Mini-Cog, MMSE and MOCA, but this was likely explained by the normal cognitive functioning of all 10 subjects at baseline. The IP was safe and well tolerated. The improvement in symptoms suggest that hBM-MSC EVs may be efficacious in the treatment of long COVID symptoms such as diminished overall quality of life, reduced functioning, and increased fatigue and pain. HBM-MSC EVs should be evaluated rigorously in randomized, controlled clinical studies as a potential novel therapy for long COVID to test the hypothesis.

Keywords: Bone marrow mesenchymal stem cell; Extracellular vesicle; Long COVID; PASC

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Introduction

Although the COVID-19 pandemic has receded, the highly mutable and transmissible nature of the SARS-CoV-2 virus presents a constant threat for continued infection. This may manifest acutely or as a chronic condition known as long COVID, or Post Acute Sequelae of COVID-19 (PASC), that lasts beyond the initial 30 days of infection and can continue for years [1-4]. The causes of long COVID are not well understood, complicating diagnosis and treatment. The condition raises policy questions, such as how best to support patients. Long COVID, affects at least 10% of all those who contract the SARS-CoV-2 virus, including those with mild to no acute symptoms upon presentation. The individual health consequences of long COVID, regardless of age, can vary from moderate and transient to severe and sustained; lost employment and increased costs of care have resulted in a substantial societal cost [1]. There are no approved pharmaceutical therapies for the treatment of long COVID although some are under investigation [5]. Currently patients must wait for the symptoms to recede, if ever, or pursue treatments that are directed at symptom relief rather than treatment of underlying disease.

The lack of targeted therapeutics underscores both the lack of understanding around the specific effects of the virus on human physiology and the highly heterogeneous nature of symptoms across multiple organ systems including immune, vascular and nervous system functions in different long COVID patients [1,6-14]. Recently, a PASC score was developed to identify patients based on symptoms [12]. Commonly reported symptoms used to develop the score included post exertional malaise, fatigue, brain fog, dizziness, gastrointestinal symptoms, palpitations, changes in sexual desire or capacity, loss of or change in smell or taste, thirst, chronic cough, chest pain, and abnormal movements. These selections were complementary to another study that identified dementia, hair loss, pressure ulcers, pulmonary fibrosis, dyspnea, pulmonary embolism, chest pain, abnormal heartbeat, malaise, and fatigue as commonly observed symptoms [13]. Six generalizable, symptom-based subclassifications were developed including neuropsychiatric, pulmonary, cardiovascular and other abnormalities [10]. Some mechanistic insight has been obtained through comparison of PASC with other virally initiated syndromes such as myalgic encephalomyelitis/chronic fatigue syndrome and postural orthostatic tachycardia syndrome [11].

Given the mechanism and symptom heterogeneity, the development of targeted therapeutics, or repurposing of drugs, to treat long COVID is delayed [1]. Overactive immune responses characterize acute COVID and highly dysregulated innate and adaptive immune systems are implicated in long COVID [1,7,8,11,15-17]. This suggests that an

immunomodulatory therapy that could minimize hyperinflammation and repolarize the immune system towards a homeostatic state may be effective against both acute and long COVID. One such therapy could arise from the documented immunomodulatory properties of mesenchymal stem cells (MSCs), in particular human bone marrow MSCs (hBM-MSCs) [18]. But, treatment with hBM-MSCs presents logistic, cost and safety challenges that limit their practical usage. Alternatively, paracrine extracellular vesicles (EVs) derived from hBM-MSCs that contain thousands of bioactive molecules can convey most of the reparative functionality of intact hBM-MSC and can be utilized much the same as an off the shelf therapeutic if manufactured, stored and applied under appropriate conditions [19,20]. The purpose of this study was primarily to evaluate the safety, and secondarily the efficacy, of intravenous (IV) infusions of a hBM-MSC EV investigational product (IP) candidate to treat subjects with a diagnosis of impairments from Long Covid. We report here the safety and preliminary efficacy results of an initial case series utilizing the IP for the treatment of long COVID. Nine patient-reported outcome measures (PROMs) addressing general quality of life (QoL), response to stress, fatigue, generalized pain and cognitive function were used to characterize responses.

Materials and Methods

Investigational product (IP)

ExoFlo™ (the IP) is an allogeneic biologic produced from human hBM-MSC currently in a phase 3 clinical trial under IND#21669 for treatment of acute respiratory distress syndrome (ARDS) due to any cause. The IP is manufactured from the banked hBM-MSCs of a single donor under cGMP conditions and according to FDA Master File protocols. Each lot of the IP meets stringent release specifications, including proteomic, mRNA and miRNA characterization. Additionally, the size and quantity of EVs and the presence of a specific surface marker expression profile are confirmed. Identity assays are combined with validated potency assays to demonstrate the mechanism of action is functional. In phase 1 and phase 2 studies it demonstrated an excellent safety profile, which has continued into the midpoint of an Expanded Access Protocol study with over 100 additional patients, and it demonstrated efficacy in patients under age 65 experiencing moderate to severe ARDS related to COVID-19 [21,22]. These studies employed up to three separate 15 mL doses of the IP administered intravenously.

Subject enrollment and approvals

The study protocol was approved by the Institute of Regenerative and Cellular Medicine IRB on 9/21/22 (Protocol number: RB-EVI-001; IRB approval number: IRCM-2022-351). Subjects meeting the inclusion and exclusion criteria (Table 1) were enrolled. Informed consent was obtained from all subjects or their legal representatives prior to any study procedure being performed. The study population was comprised of 10 subjects of any racial or ethnic origin, balanced by gender and over 21 years of age, who had Long Covid and who complained of chronic fatigue and mental status changes.

Study design

The study was a prospective non-randomized study conducted at the office of Richard Bligh, MD. The subjects received one 15 mL IV infusion of the IP (15 mL of the IP was mixed with 85 mL sterile saline for a total of 100 mL of infusion), and this was repeated one week later. Dose amount and frequency of the IP was based on the dosing rationale used in studies with COVID-19 associated ARDS patients in which no serious adverse events were attributed to the IP [21,22]. Safety evaluation of all adverse events and complications was reported by the treating physician who performed daily safety evaluations during the one week infusion period.

All candidates for this study must meet ALL of the following Inclusion Criteria to be eligible for enrollment:	
INCLUSION CRITERIA	1. Voluntary signature of the approved Informed Consent by the patient or legal representative. Subject must be over 21 years old.
	2. A diagnosis of Long Covid-19.
Candidates who meet ANY of the following Exclusion Criteria at the time of the study procedure are NOT eligible for enrollment in the study:	
EXCLUSION CRITERIA	1. The subject is unable to conform to the study protocol follow-up procedures and visits.
	2. The subject has major risk factors such as a history of narcotic abuse, paucity of family support, unemployed, history of previous physical or mental abuse or severe medical comorbidities beyond Long Covid-19.
	3. Patients with any other auto-immune disorder.
	4. Any patient with a previous positive test for tuberculosis.
	5. Patients with a history of chronic steroid use.
	6. Any patient with a history of any cancer.
	7. Any patient requiring treatment for high blood pressure.
	8. Any patient requiring treatment for diabetes.
	9. Any patient with a history of cardiac, liver or renal disease.
	10. Any patient positive for HIV.
	11. Patients who have received any other investigational drugs for treatment.
	12. Any patient felt not to be a suitable study patient by the principal Investigator.

Table 1: Subject Inclusion and Exclusion Criteria.

Quality of life assessments and other metrics

A battery of disease independent and disease dependent patient reported outcome measures (PROMs) to measure quality of life (QoL), fatigue, pain and cognitive functioning were administered before treatment with the IP (Week 0) and at intervals (Weeks 3, 6, 12, 24) after treatment.

Assessments before treatment (Week 0) were given in person while the remainder were performed either in person or by videoconference according to the subject's availability. The 9 assessments were as follows.

PROMIS-Global Health – Patient-Reported Outcomes Measurement Information System. PROMIS

PROMIS Health Organization resulted from an NIH sponsored initiative to develop a PROM system to assess an individual's physical, mental, and social health that is efficient, flexible, reproducible and precise in the measurement of generic symptoms [23]. PROMIS scores herein are separated into *Mental* and *Physical Health* total scores. A *T-score* metric is also reported in which 50 is the mean of the reference US general population and 10 units is the standard deviation (SD).

EQ-5D-5L – A short, easily administered, robust, reliable and responsive generic QoL questionnaire developed by the EuroQol Group that assess five parameters: mobility, self-care, usual activities, pain and discomfort, and anxiety and depression [EQ-5D (euroqol.org)] [24]. It also includes a single visual analog scale to indicate the subject's health status, and these are reported separately herein as an *Overall Health Score*.

SF-3C – 36-Item Short Form Health Survey. The SF-36 is a set of generic, coherent, and easily administered quality-of-life measures assessed by questionnaire developed by the Rand Corporation as part of an effort to explain variations in patient outcomes [36-Item Short Form Survey (SF-36) | RAND]. Eight scaled scores that assesses vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning, and mental health or emotional wellbeing are reduced to a total score. SF-36 was used to assess the effects of COVID-19 on QoL [25].

IES-R – Impact of Event Scale-Revised. Originally developed to sensitively measure intrusion and avoidance responses following any type of stressful life event, IES can be used repeatedly to follow the response to a specific event over time [26]. It was revised (IES-R) to also address persistent hyperarousal, the third major symptom cluster of posttraumatic stress disorder (PTSD) [27].

PCFS – Post-COVID-19 Functional Status Scale. A PROM designed to complement other metrics, evaluate the ultimate consequences of COVID-19 on functional status and help identify patients undergoing incomplete recovery from SARS-CoV-2 infection [28]. It consists of grades 0–4 based on yes/no responses to four component questions. Grade 0 reflects the absence of any functional limitation and grade 4 reflects requiring assistance with activities of daily living (severe functional limitations).

FSS – Fatigue Severity Scale. Originally developed to describe fatigue in multiple sclerosis (MS) and systemic lupus erythematosus (SLE) patients, FSS is an internally consistent metric that correlates well with visual analog measures, is largely independent of self-reported depressive symptoms, and could differentiate fatigue associated with MS from that associated with SLE [29,30]. It consists of nine items scored on a seven-point Likert-type scale ranging from strongly disagree to strongly agree. The nine items are combined into a total score calculated as the average of the individual item responses.

Mini-Cog – a simple, brief and easily administered test that includes recalling a three-word list of objects and drawing a clock to discriminate demented from non-demented individuals [31].

MMSE – Mini-Mental State Examination. A 7-to-10-minute screening tool used to detect and quantify the severity of cognitive impairment that includes naming the current date, counting backward, and identifying everyday objects like a pencil or watch [32].

MOCA – Montreal Cognitive Assessment. A 10-to-15-minute screening tool for detection of mild cognitive impairment (MCI) with superior sensitivity as compared to the MMSE that includes memorizing a short list of words, identifying a picture of an animal, and copying a drawing of a shape or object [33,34].

Statistical analysis

All data sets were subjected to repeated measures one way ANOVA with a Geisser-Greenhouse correction using GraphPad Prism 9.5.1 software. Subsequent pairwise comparisons between Week 0 and subsequent weeks for each assessment metric employed a parametric paired t-test assuming a Gaussian distribution.

For all plotted results *p<0.05, **p<0.01, ***p<0.001,****p<0.0001 and ns=not significant.

Results

Demographics

Subjects (6 female, 4 male) ranged in age from 39 to 80 years with a mean age of 60 (Table 2). Weights ranged from 122 to 252 pounds with a mean of 171 pounds. Subject vital signs at the two treatment visits are shown in (Table 3) and were generally consistent at each time with no deviations resulting in an adverse or serious adverse event.

	Gender	Age	Weight
Subject 1	M	56	252
Subject 2	M	51	145.8
Subject 3	F	76	138
Subject 4	F	80	125
Subject 5	F	67	134
Subject 6	M	66	227.3
Subject 7	F	67	197.2
Subject 8	M	59	235
Subject 9	F	41	135
Subject 10	F	39	122.00
Means		60.2	171.13

Table 2: Subject demographics.

	Blood Pressure		Heart Rate		O2 Saturation	
	first treatment	second treatment	first treatment	second treatment	first treatment	second treatment
Subject 1	142/90	157/94	74	62	94	97
Subject 2	129/92	136/85	94	72	98	96
Subject 3	158/61	168/72	60	56	99	98
Subject 4	105/70	132/89	71	72	99	99
Subject 5	97/67	94/60	76	69	99	99
Subject 6	140/84	148/85	70	66	95	97
Subject 7	142/88	132/75	77	87	97	97
Subject 8	130/76	124/91	88	95	99	99
Subject 9	124/83	110/70	64	73	97	99
Subject10	101/76	104/64	85	74	98	99

Table 3: Subject Vital Statistics Prior to Each Treatment.

Safety

No adverse events or serious adverse events related to IP were reported during the infusion periods or up to the 24 week follow-up.

QoL and stress

The PROMIS Mental Health and Physical Health scores for the subject pool show significant improvements by week 3 that are sustained for 24 weeks (Figures 1A&1B) and all subjects had progressed by week 24 (Figures 1C&1D) (Figures 1E&1F). Compared to the US population

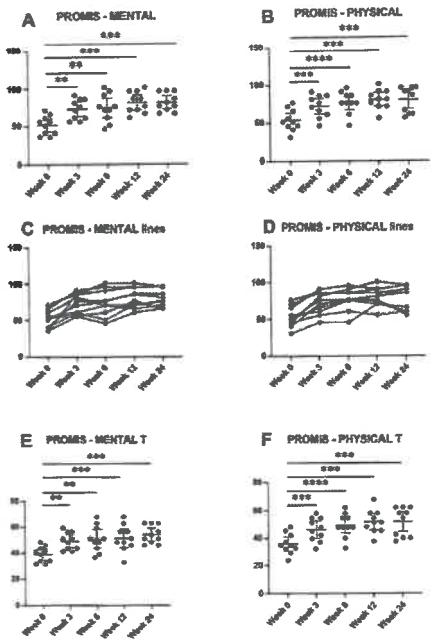

Figure 1: PROMIS scores. Panels A-D show PROMIS scores over the study period. Thick center bars are means and whiskers are 95% confidence intervals. P values derived from paired t test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns = not significant (p>0.05). Panels C and D are continuous plots of individual subject scores over time. Panels E and F show the T-scores in comparison to the US population reference set.

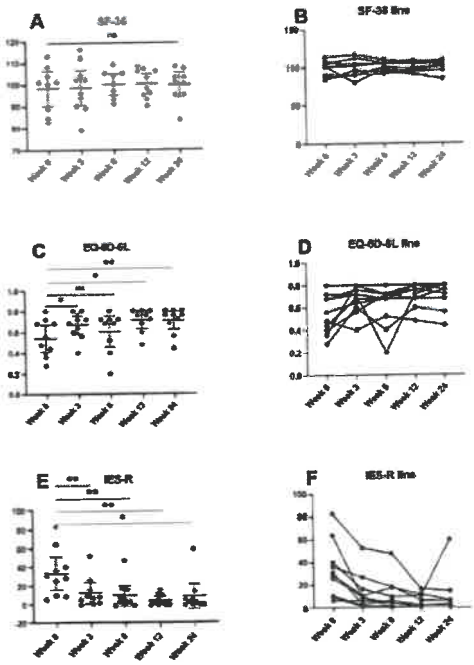

Figure 2: SF-36, EQ-SD and IES-R scores over the study period. Thick center bars are means and whiskers are 95% confidence intervals. P values derived from paired t test: *p<0.05, **p<0.01, ns = not significant (p>0.05). Panels B, D and F are continuous plots of individual subject scores over time.

reference set T-score (mean=50), the group had progressed from mean values of 38.9 to 53.8 for Mental Health by week 24 and from 36.0 to 51.5 for Physical Health placing them within one standard deviation (40-60) of the reference group.

EQ-5D-5L and IES-R scores achieved significance within the first follow-up, and significant improvements were measured at week 24 (Figure 2). All subjects improved in IES-R by week 12 and the group showed significant improvement at week 24, but one worsened by week 24. The group exhibited significant improvement in EQ-5D-5L scores by week 24. No significant effect on the SF- 36 scores was evident.

Other

Figure 3 shows the results of the PCFS, FSS, Average Pain (queried in PROMIS) and Overall Health (EQ-5D-5L) metrics. All four group scores improved significantly within three weeks and those improvements were sustained or improved over 24 weeks.By week 24 all subjects improved in PCFS, all but one improved in Pain, and all but one improved in Overall Health.

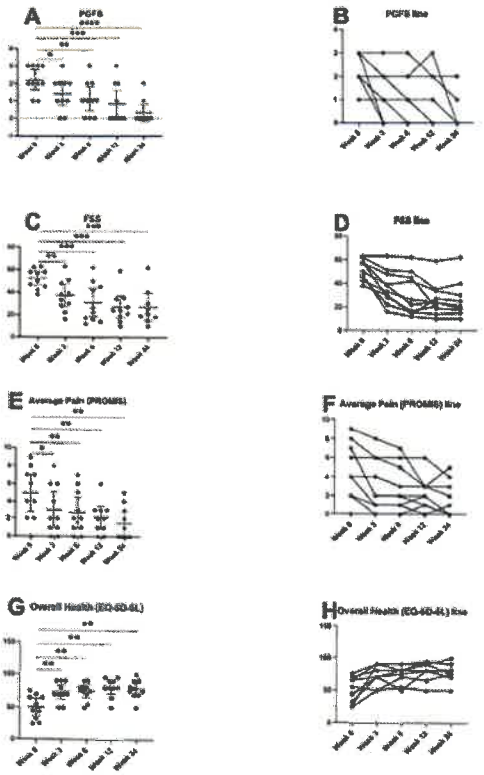

Figure 3: PCFS, FSS, Average Pain and Overall Health scores over the study period. Thick center bars are means and whiskers are 95% confidence intervals.

P values derived from paired t test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns not significant (p>0.05). Panels B, D, F and H are continuous plots of individual subject scores over time.

Cognition

None of the cognitive function tests exhibited improvements except for a significant improvement in MiniCog scores at week 24 (Figure 4).

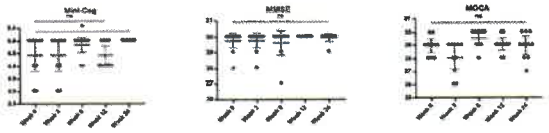

Figure 4: Mini-Cog, MMSE and MOCA scores over the study period. Thick center bars are means and whiskers are 95% confidence intervals.

P values derived from paired t test: *p<0.05, ns = not significant (p>0.05).

Discussion

Patients suffering from long COVID have no approved therapeutic options available and treatment is palliative. This prospective study was intended to assess the safety and explore the potential efficacy of IV administration of a hBM-MSC EV preparation for the treatment of long COVID. No adverse or serious adverse events either occurred or were attributable to the IP, and significant improvements in subject symptoms were evident across most tests in a battery of nine PROMs as compared to baseline values. IV administration of a similar dosing regimen of the IP as used here has demonstrated an excellent safety profile in phase 1 and phase 2 studies of treatment of acute COVID-19 associated ARDS [21,22,35]. An ongoing Expanded Access Protocol on over 100 patients to date also shows excellent safety. Thus, the IP appears suitable for further efficacy studies in long COVID patients. This study has limitation in that it did not include a control, untreated arm and there was no randomization of subjects, so the influence of unknown variables and bias cannot be quantified. Nor did it explore different dose regimens, so the influence of variable exposure to the IP is not clarified. And, the data spans 24 weeks from the time of the first treatment making extended durability of the responses uncertain. But, it employed multiple PROMs to assess the subjects' conditions following the treatment, the adult study group represented a balance of gender, weight and age, and statistically significant and sustained improvement was observed for many of the metrics at the earliest time point as compared to baseline, and this was sustained throughout. Given the disruption to innate and adaptive immune responses (e.g., mast cell activation, neutrophil dysfunction, uncontrolled inflammation), vascular system function (e.g., coagulopathy) and neurological capacities (e.g., cognitive dysfunction, fatigue, memory lapse) in long COVID patients and given the immunomodulatory and multi-organ regenerative capacity of hBM-MSC, we sought to interrogate the potential of an EV preparation obtained from hBM-MSC to positively affect long COVID. Except for the SF-36 metric, two of the three general QoL PROMs showed sustained subject improvement within three weeks of the first treatment. The reason(s) for the difference between the SF-36 mechanism, the EQ-5D-5L and the PROMIS metric is unclear, but there was a decrease in variance of subject scores in SF-36 by week 12. There was also continuous, and highly significant improvement in the IES-R scores across the group suggesting that their ability to manage intrusion, avoidance and hyperarousal responses to the stress of long COVID was improved.

Significant improvement amongst the subjects was observed for the COVID specific PCFS metric that quantifies long COVID symptom severity, suggesting that their symptoms were diminishing over time. Similarly, significant ($p<0.01$) improvement in the FSS scores occurred within three weeks and this improved further over time suggesting that at least one very common specific symptom of

long COVID, fatigue, may be successfully targeted. Scores on the generalized pain metric, another symptom experienced by many patients, were also significantly reduced by the third week and this was sustained and improved over the 24-week study. This may be due to the documented ability of hBM-MSC and their EVs to modulate the immune system away from a proinflammatory response, which can otherwise promote neuropathic pain [36]. Finally, the Overall Health Score results were consistent with the QoL and specific symptom scores in that a highly significant improvement was rapid and sustained .

In PCFS and FSS there was one subject who was not highly responsive to the treatment. This indicates that in larger controlled studies there is likely to be some patient response heterogeneity that should be addressed by appropriately powering the study to illuminate effectiveness overall.

With exception of the MiniCog at week 24, the cognitive function metrics showed no statistical improvement, most likely because no subjects exhibited impairment as measured by the three metrics at the baseline tests. Hence, there was little room within any of the scales to indicate improvement. Future RCTs would need to screen subjects for mild cognitive impairment to ensure that enough such subjects are enrolled to enable detection of any possible positive effect on cognitive impairment.

The mechanism(s) mediated by hBM-MSC EVs possibly responsible for the observed results may be varied due to the wide array of bioactive molecules within EVs and the pleiotropic activities of an EV population [19,20]. Overactive immune responses characterize acute COVID, and highly dysregulated innate and adaptive immune systems and neuroinflammatory processes are implicated in long COVID [1,8,11]. The documented capacity of cytokines, miRNA and other bioactive molecules contained in hMSC EVs to modulate such inflammatory processes likely contribute to the effects seen in this study [18,37,38]. EVs are also able to favorably modulate pain syndromes, consistent with results here [36,39]. Additionally, the angiogenic potential of hBM-MSC EVs may contribute to recovery by improving oxygen delivery to compromised tissues [40,41].

Symptoms of long COVID are apparent for years, and the CDC Household Pulse Survey indicates that the percentage of all US adults that experience any activity limitations from long COVID was 5.6% in March 2024 (95% CI = 5.3-5.9), essentially identical to the 5.9% in September 2022 (95% CI = 5.4-6.4) [2-4,42]. These observations indicate that long COVID symptoms do not resolve rapidly and the rapid and sustained improvement results presented here support the possibility that the IP may help long COVID patients achieve a greater QoL. Further randomized controlled study is appropriate to confirm this possibility. Response heterogeneity amongst the subjects in terms of achieving resolution of certain symptoms rapidly suggests that future studies should explore additional dose amount and frequency options to determine if heterogeneous responsiveness can be overcome. Such response heterogeneity also presents an opportunity, in larger controlled studies, to understand more about the underlying pathologies of long COVID and how different mechanisms might be targeted by this and other approaches.

Conclusion

The hBM-MSC EV IP was safe following IV infusion of two doses in long COVID subjects. Improvements in quality of life, fatigue and pain metrics indicate that hBM-MSC EVs should be evaluated further as a potential novel and effective treatment for long COVID.

Author Contributions

Richard Bligh performed study design, patient recruitment, patient interaction, data acquisition/writing Robert Besancenez performed patient interaction and data acquisition.

Ethical Disclosure

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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Author Disclosures

John T. Ransom is Sr. Medical Writer at Direct Biologics.

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Research Article

Safety and Efficacy of Bone Marrow Mesenchymal Stem Cell Extracellular Vesicles in Long COVID Patients: A Case Series

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Abstract

Long COVID, or Post Acute Sequelae of COVID-19 (PASC), is a prolonged, debilitating syndrome that follows acute SARS-CoV-2 infection in >10% of cases. With immunomodulatory and regenerative properties, human bone marrow mesenchymal stem cell derived extracellular vesicles (hBM-MSC EVs) may present a new therapeutic option. To explore this treatment option, we performed a prospective IRB safety study with an advanced BM-MSC EV investigational product (IP) and measured subject status using patient-reported outcome measures (PROMs). Ten subjects with confirmed long COVID symptoms received two intravenous 15 mL doses of IP one week apart. Safety events and subject status were monitored over six months. No serious adverse events occurred. Statistically significant improvements, as compared to baseline, were observed for the following PROMs as early as three weeks after first infusion and were sustained for six months: PROMIS (mental, physical, average pain), EQ-5D-5L, IES-R, PCFS, and FSS. No improvement was detected by SF-36. Nor was improvement indicated by the cognitive assessments Mini-Cog, MMSE and MOCA, but this was likely explained by the normal cognitive functioning of all 10 subjects at baseline. The IP was safe and well tolerated. The improvement in symptoms suggest that hBM-MSC EVs may be efficacious in the treatment of long COVID symptoms such as diminished overall quality of life, reduced functioning, and increased fatigue and pain. HBM-MSC EVs should be evaluated rigorously in randomized, controlled clinical studies as a potential novel therapy for long COVID to test the hypothesis.

Keywords: Bone marrow mesenchymal stem cell; Extracellular vesicle; Long COVID; PASC

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Introduction

Although the COVID-19 pandemic has receded, the highly mutable and transmissible nature of the SARS-CoV-2 virus presents a constant threat for continued infection. This may manifest acutely or as a chronic condition known as long COVID, or Post Acute Sequelae of COVID-19 (PASC), that lasts beyond the initial 30 days of infection and can continue for years [1-4]. The causes of long COVID are not well understood, complicating diagnosis and treatment. The condition raises policy questions, such as how best to support patients. Long COVID, affects at least 10% of all those who contract the SARS-CoV-2 virus, including those with mild to no acute symptoms upon presentation. The individual health consequences of long COVID, regardless of age, can vary from moderate and transient to severe and sustained; lost employment and increased costs of care have resulted in a substantial societal cost [1]. There are no approved pharmaceutical therapies for the treatment of long COVID although some are under investigation [5]. Currently patients must wait for the symptoms to recede, if ever, or pursue treatments that are directed at symptom relief rather than treatment of underlying disease.

The lack of targeted therapeutics underscores both the lack of understanding around the specific effects of the virus on human physiology and the highly heterogeneous nature of symptoms across multiple organ systems including immune, vascular and nervous system functions in different long COVID patients [1,6-14]. Recently, a PASC score was developed to identify patients based on symptoms [12]. Commonly reported symptoms used to develop the score included post exertional malaise, fatigue, brain fog, dizziness, gastrointestinal symptoms, palpitations, changes in sexual desire or capacity, loss of or change in smell or taste, thirst, chronic cough, chest pain, and abnormal movements. These selections were complementary to another study that identified dementia, hair loss, pressure ulcers, pulmonary fibrosis, dyspnea, pulmonary embolism, chest pain, abnormal heartbeat, malaise, and fatigue as commonly observed symptoms [13]. Six generalizable, symptom-based subclassifications were developed including neuropsychiatric, pulmonary, cardiovascular and other abnormalities [10]. Some mechanistic insight has been obtained through comparison of PASC with other virally initiated syndromes such as myalgic encephalomyelitis/chronic fatigue syndrome and postural orthostatic tachycardia syndrome [11].

Given the mechanism and symptom heterogeneity, the development of targeted therapeutics, or repurposing of drugs, to treat long COVID is delayed [1]. Overactive immune responses characterize acute COVID and highly dysregulated innate and adaptive immune systems are implicated in long COVID [1,7,8,11,15-17]. This suggests that an

immunomodulatory therapy that could minimize hyperinflammation and repolarize the immune system towards a homeostatic state may be effective against both acute and long COVID. One such therapy could arise from the documented immunomodulatory properties of mesenchymal stem cells (MSCs), in particular human bone marrow MSCs (hBM-MSCs) [18]. But, treatment with hBM-MSCs presents logistic, cost and safety challenges that limit their practical usage. Alternatively, paracrine extracellular vesicles (EVs) derived from hBM-MSCs that contain thousands of bioactive molecules can convey most of the reparative functionality of intact hBM-MSC and can be utilized much the same as an off the shelf therapeutic if manufactured, stored and applied under appropriate conditions [19,20]. The purpose of this study was primarily to evaluate the safety, and secondarily the efficacy, of intravenous (IV) infusions of a hBM-MSC EV investigational product (IP) candidate to treat subjects with a diagnosis of impairments from Long Covid. We report here the safety and preliminary efficacy results of an initial case series utilizing the IP for the treatment of long COVID. Nine patient-reported outcome measures (PROMs) addressing general quality of life (QoL), response to stress, fatigue, generalized pain and cognitive function were used to characterize responses.

Materials and Methods

Investigational product (IP)

ExoFlo™ (the IP) is an allogeneic biologic produced from human hBM-MSC currently in a phase 3 clinical trial under IND#21669 for treatment of acute respiratory distress syndrome (ARDS) due to any cause. The IP is manufactured from the banked hBM-MSCs of a single donor under cGMP conditions and according to FDA Master File protocols. Each lot of the IP meets stringent release specifications, including proteomic, mRNA and miRNA characterization. Additionally, the size and quantity of EVs and the presence of a specific surface marker expression profile are confirmed. Identity assays are combined with validated potency assays to demonstrate the mechanism of action is functional. In phase 1 and phase 2 studies it demonstrated an excellent safety profile, which has continued into the midpoint of an Expanded Access Protocol study with over 100 additional patients, and it demonstrated efficacy in patients under age 65 experiencing moderate to severe ARDS related to COVID-19 [21,22]. These studies employed up to three separate 15 mL doses of the IP administered intravenously.

Subject enrollment and approvals

The study protocol was approved by the Institute of Regenerative and Cellular Medicine IRB on 9/21/22 (Protocol number: RB-EVI-001; IRB approval number: IRCM-2022-351). Subjects meeting the inclusion and exclusion criteria (Table 1) were enrolled. Informed consent was obtained from all subjects or their legal representatives prior to any study procedure being performed. The study population was comprised of 10 subjects of any racial or ethnic origin, balanced by gender and over 21 years of age, who had Long Covid and who complained of chronic fatigue and mental status changes.

Study design

The study was a prospective non-randomized study conducted at the office of Richard Bligh, MD. The subjects received one 15 mL IV infusion of the IP (15 mL of the IP was mixed with 85 mL sterile saline for a total of 100 mL of infusion), and this was repeated one week later. Dose amount and frequency of the IP was based on the dosing rationale used in studies with COVID-19 associated ARDS patients in which no serious adverse events were attributed to the IP [21,22]. Safety evaluation of all adverse events and complications was reported by the treating physician who performed daily safety evaluations during the one week infusion period.

All candidates for this study must meet ALL of the following Inclusion Criteria to be eligible for enrollment:	
INCLUSION CRITERIA	1. Voluntary signature of the approved Informed Consent by the patient or legal representative. Subject must be over 21 years old.
	2. A diagnosis of Long Covid-19.
Candidates who meet ANY of the following Exclusion Criteria at the time of the study procedure are NOT eligible for enrollment in the study:	
EXCLUSION CRITERIA	1. The subject is unable to conform to the study protocol follow-up procedures and visits.
	2. The subject has major risk factors such as a history of narcotic abuse, paucity of family support, unemployed, history of previous physical or mental abuse or severe medical comorbidities beyond Long Covid-19.
	3. Patients with any other auto-immune disorder.
	4. Any patient with a previous positive test for tuberculosis.
	5. Patients with a history of chronic steroid use.
	6. Any patient with a history of any cancer.
	7. Any patient requiring treatment for high blood pressure.
	8. Any patient requiring treatment for diabetes.
	9. Any patient with a history of cardiac, liver or renal disease.
	10. Any patient positive for HIV.
	11. Patients who have received any other investigational drugs for treatment.
	12. Any patient felt not to be a suitable study patient by the principal Investigator.

Table 1: Subject Inclusion and Exclusion Criteria.

Quality of life assessments and other metrics

A battery of disease independent and disease dependent patient reported outcome measures (PROMs) to measure quality of life (QoL), fatigue, pain and cognitive functioning were administered before treatment with the IP (Week 0) and at intervals (Weeks 3, 6, 12, 24) after treatment.

Assessments before treatment (Week 0) were given in person while the remainder were performed either in person or by videoconference according to the subject's availability. The 9 assessments were as follows.

PROMIS-Global Health – Patient-Reported Outcomes Measurement Information System. PROMIS

PROMIS Health Organization resulted from an NIH sponsored initiative to develop a PROM system to assess an individual's physical, mental, and social health that is efficient, flexible, reproducible and precise in the measurement of generic symptoms [23]. PROMIS scores herein are separated into *Mental* and *Physical Health* total scores. A *T-score* metric is also reported in which 50 is the mean of the reference US general population and 10 units is the standard deviation (SD).

EQ-5D-5L – A short, easily administered, robust, reliable and responsive generic QoL questionnaire developed by the EuroQol Group that assess five parameters: mobility, self-care, usual activities, pain and discomfort, and anxiety and depression [EQ-5D (euroqol.org)] [24]. It also includes a single visual analog scale to indicate the subject's health status, and these are reported separately herein as an *Overall Health Score*.

SF-3C – 36-Item Short Form Health Survey. The SF-36 is a set of generic, coherent, and easily administered quality-of-life measures assessed by questionnaire developed by the Rand Corporation as part of an effort to explain variations in patient outcomes [36-Item Short Form Survey (SF-36) | RAND]. Eight scaled scores that assesses vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning, and mental health or emotional wellbeing are reduced to a total score. SF-36 was used to assess the effects of COVID-19 on QoL [25].

IES-R – Impact of Event Scale-Revised. Originally developed to sensitively measure intrusion and avoidance responses following any type of stressful life event, IES can be used repeatedly to follow the response to a specific event over time [26]. It was revised (IES-R) to also address persistent hyperarousal, the third major symptom cluster of posttraumatic stress disorder (PTSD) [27].

PCFS – Post-COVID-19 Functional Status Scale. A PROM designed to complement other metrics, evaluate the ultimate consequences of COVID-19 on functional status and help identify patients undergoing incomplete recovery from SARS-CoV-2 infection [28]. It consists of grades 0–4 based on yes/no responses to four component questions. Grade 0 reflects the absence of any functional limitation and grade 4 reflects requiring assistance with activities of daily living (severe functional limitations).

FSS – Fatigue Severity Scale. Originally developed to describe fatigue in multiple sclerosis (MS) and systemic lupus erythematosus (SLE) patients, FSS is an internally consistent metric that correlates well with visual analog measures, is largely independent of self-reported depressive symptoms, and could differentiate fatigue associated with MS from that associated with SLE [29,30]. It consists of nine items scored on a seven-point Likert-type scale ranging from strongly disagree to strongly agree. The nine items are combined into a total score calculated as the average of the individual item responses.

Mini-Cog – a simple, brief and easily administered test that includes recalling a three-word list of objects and drawing a clock to discriminate demented from non-demented individuals [31].

MMSE – Mini-Mental State Examination. A 7-to-10-minute screening tool used to detect and quantify the severity of cognitive impairment that includes naming the current date, counting backward, and identifying everyday objects like a pencil or watch [32].

MOCA – Montreal Cognitive Assessment. A 10-to-15-minute screening tool for detection of mild cognitive impairment (MCI) with superior sensitivity as compared to the MMSE that includes memorizing a short list of words, identifying a picture of an animal, and copying a drawing of a shape or object [33,34].

Statistical analysis

All data sets were subjected to repeated measures one way ANOVA with a Geisser-Greenhouse correction using GraphPad Prism 9.5.1 software. Subsequent pairwise comparisons between Week 0 and subsequent weeks for each assessment metric employed a parametric paired t-test assuming a Gaussian distribution.

For all plotted results *p<0.05, **p<0.01, ***p<0.001,****p<0.0001 and ns=not significant.

Results

Demographics

Subjects (6 female, 4 male) ranged in age from 39 to 80 years with a mean age of 60 (Table 2). Weights ranged from 122 to 252 pounds with a mean of 171 pounds. Subject vital signs at the two treatment visits are shown in (Table 3) and were generally consistent at each time with no deviations resulting in an adverse or serious adverse event.

	Gender	Age	Weight
Subject 1	M	56	252
Subject 2	M	51	145.8
Subject 3	F	76	138
Subject 4	F	80	125
Subject 5	F	67	134
Subject 6	M	66	227.3
Subject 7	F	67	197.2
Subject 8	M	59	235
Subject 9	F	41	135
Subject 10	F	39	122.00
Means		60.2	171.13

Table 2: Subject demographics.

	Blood Pressure		Heart Rate		O2 Saturation	
	first treatment	second treatment	first treatment	second treatment	first treatment	second treatment
Subject 1	142/90	157/94	74	62	94	97
Subject 2	129/92	136/85	94	72	98	96
Subject 3	158/61	168/72	60	56	99	98
Subject 4	105/70	132/89	71	72	99	99
Subject 5	97/67	94/60	76	69	99	99
Subject 6	140/84	148/85	70	66	95	97
Subject 7	142/88	132/75	77	87	97	97
Subject 8	130/76	124/91	88	95	99	99
Subject 9	124/83	110/70	64	73	97	99
Subject10	101/76	104/64	85	74	98	99

Table 3: Subject Vital Statistics Prior to Each Treatment.

Safety

No adverse events or serious adverse events related to IP were reported during the infusion periods or up to the 24 week follow-up.

QoL and stress

The PROMIS Mental Health and Physical Health scores for the subject pool show significant improvements by week 3 that are sustained for 24 weeks (Figures 1A&1B) and all subjects had progressed by week 24 (Figures 1C&1D) (Figures 1E&1F). Compared to the US population

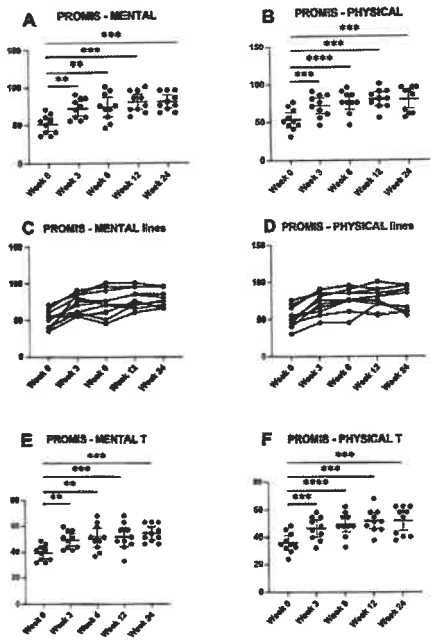

Figure 1: PROMIS scores. Panels A-D show PROMIS scores over the study period. Thick center bars are means and whiskers are 95% confidence intervals. P values derived from paired t test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns = not significant (p>0.05). Panels C and D are continuous plots of individual subject scores over time. Panels E and F show the T-scores in comparison to the US population reference set.

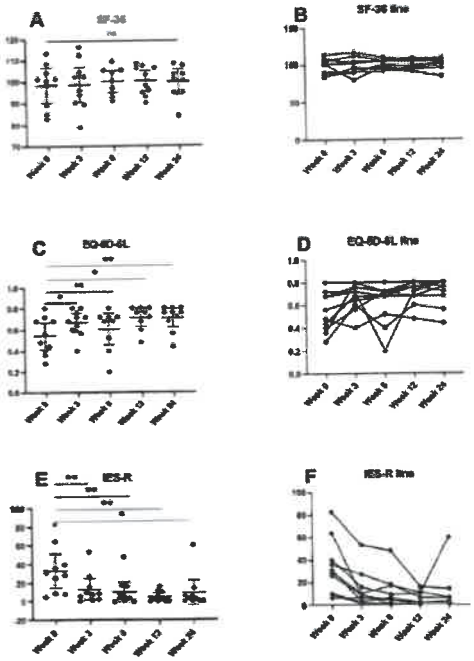

Figure 2: SF-36, EQ-5D and IES-R scores over the study period. Thick center bars are means and whiskers are 95% confidence intervals. P values derived from paired t test: *p<0.05, **p<0.01, ns = not significant (p>0.05). Panels B, D and F are continuous plots of individual subject scores over time.

reference set T-score (mean=50), the group had progressed from mean values of 38.9 to 53.8 for Mental Health by week 24 and from 36.0 to 51.5 for Physical Health placing them within one standard deviation (40-60) of the reference group.

EQ-5D-5L and IES-R scores achieved significance within the first follow-up, and significant improvements were measured at week 24 (Figure 2). All subjects improved in IES-R by week 12 and the group showed significant improvement at week 24, but one worsened by week 24. The group exhibited significant improvement in EQ-5D-5L scores by week 24. No significant effect on the SF- 36 scores was evident.

Other

Figure 3 shows the results of the PCFS, FSS, Average Pain (queried in PROMIS) and Overall Health (EQ-5D-5L) metrics. All four group scores improved significantly within three weeks and those improvements were sustained or improved over 24 weeks.By week 24 all subjects improved in PCFS, all but one improved in Pain, and all but one improved in Overall Health.

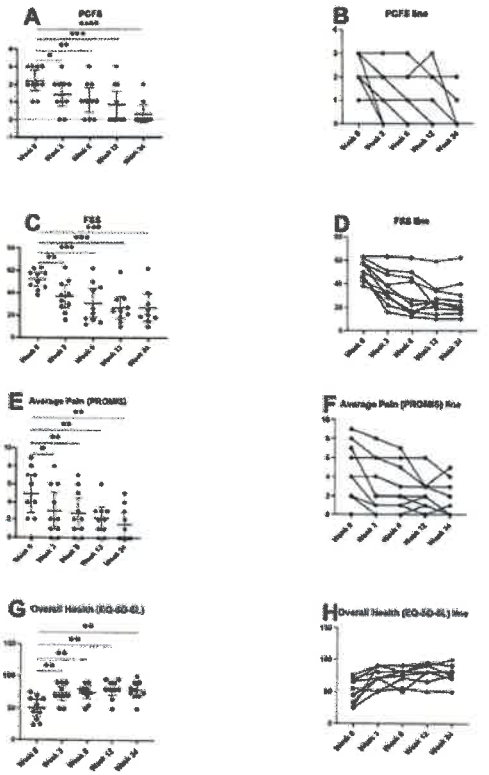

Figure 3: PCFS, FSS, Average Pain and Overall Health scores over the study period. Thick center bars are means and whiskers are 95% confidence intervals.

P values derived from paired t test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns not significant (p>0.05). Panels B, D, F and H are continuous plots of individual subject scores over time.

Cognition

None of the cognitive function tests exhibited improvements except for a significant improvement in MiniCog scores at week 24 (Figure 4).

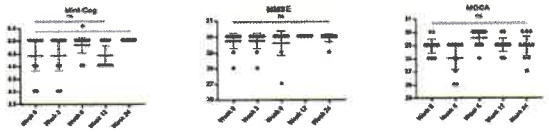

Figure 4: Mini-Cog, MMSE and MOCA scores over the study period. Thick center bars are means and whiskers are 95% confidence intervals.

P values derived from paired t test: *p<0.05, ns = not significant (p>0.05).

Discussion

Patients suffering from long COVID have no approved therapeutic options available and treatment is palliative. This prospective study was intended to assess the safety and explore the potential efficacy of IV administration of a hBM-MSC EV preparation for the treatment of long COVID. No adverse or serious adverse events either occurred or were attributable to the IP, and significant improvements in subject symptoms were evident across most tests in a battery of nine PROMs as compared to baseline values. IV administration of a similar dosing regimen of the IP as used here has demonstrated an excellent safety profile in phase 1 and phase 2 studies of treatment of acute COVID-19 associated ARDS [21,22,35]. An ongoing Expanded Access Protocol on over 100 patients to date also shows excellent safety. Thus, the IP appears suitable for further efficacy studies in long COVID patients. This study has limitation in that it did not include a control, untreated arm and there was no randomization of subjects, so the influence of unknown variables and bias cannot be quantified. Nor did it explore different dose regimens, so the influence of variable exposure to the IP is not clarified. And, the data spans 24 weeks from the time of the first treatment making extended durability of the responses uncertain. But, it employed multiple PROMs to assess the subjects' conditions following the treatment, the adult study group represented a balance of gender, weight and age, and statistically significant and sustained improvement was observed for many of the metrics at the earliest time point as compared to baseline, and this was sustained throughout. Given the disruption to innate and adaptive immune responses (e.g., mast cell activation, neutrophil dysfunction, uncontrolled inflammation), vascular system function (e.g., coagulopathy) and neurological capacities (e.g., cognitive dysfunction, fatigue, memory lapse) in long COVID patients and given the immunomodulatory and multi-organ regenerative capacity of hBM-MSC, we sought to interrogate the potential of an EV preparation obtained from hBM-MSC to positively affect long COVID. Except for the SF-36 metric, two of the three general QoL PROMs showed sustained subject improvement within three weeks of the first treatment. The reason(s) for the difference between the SF-36 mechanism, the EQ-5D-5L and the PROMIS metric is unclear, but there was a decrease in variance of subject scores in SF-36 by week 12. There was also continuous, and highly significant improvement in the IES-R scores across the group suggesting that their ability to manage intrusion, avoidance and hyperarousal responses to the stress of long COVID was improved.

Significant improvement amongst the subjects was observed for the COVID specific PCFS metric that quantifies long COVID symptom severity, suggesting that their symptoms were diminishing over time. Similarly, significant ($p<0.01$) improvement in the FSS scores occurred within three weeks and this improved further over time suggesting that at least one very common specific symptom of

long COVID, fatigue, may be successfully targeted. Scores on the generalized pain metric, another symptom experienced by many patients, were also significantly reduced by the third week and this was sustained and improved over the 24-week study. This may be due to the documented ability of hBM-MSC and their EVs to modulate the immune system away from a proinflammatory response, which can otherwise promote neuropathic pain [36]. Finally, the Overall Health Score results were consistent with the QoL and specific symptom scores in that a highly significant improvement was rapid and sustained .

In PCFS and FSS there was one subject who was not highly responsive to the treatment. This indicates that in larger controlled studies there is likely to be some patient response heterogeneity that should be addressed by appropriately powering the study to illuminate effectiveness overall.

With exception of the MiniCog at week 24, the cognitive function metrics showed no statistical improvement, most likely because no subjects exhibited impairment as measured by the three metrics at the baseline tests. Hence, there was little room within any of the scales to indicate improvement. Future RCTs would need to screen subjects for mild cognitive impairment to ensure that enough such subjects are enrolled to enable detection of any possible positive effect on cognitive impairment.

The mechanism(s) mediated by hBM-MSC EVs possibly responsible for the observed results may be varied due to the wide array of bioactive molecules within EVs and the pleiotropic activities of an EV population [19,20]. Overactive immune responses characterize acute COVID, and highly dysregulated innate and adaptive immune systems and neuroinflammatory processes are implicated in long COVID [1,8,11]. The documented capacity of cytokines, miRNA and other bioactive molecules contained in hMSC EVs to modulate such inflammatory processes likely contribute to the effects seen in this study [18,37,38]. EVs are also able to favorably modulate pain syndromes, consistent with results here [36,39]. Additionally, the angiogenic potential of hBM-MSC EVs may contribute to recovery by improving oxygen delivery to compromised tissues [40,41].

Symptoms of long COVID are apparent for years, and the CDC Household Pulse Survey indicates that the percentage of all US adults that experience any activity limitations from long COVID was 5.6% in March 2024 (95% CI = 5.3-5.9), essentially identical to the 5.9% in September 2022 (95% CI = 5.4-6.4) [2-4,42]. These observations indicate that long COVID symptoms do not resolve rapidly and the rapid and sustained improvement results presented here support the possibility that the IP may help long COVID patients achieve a greater QoL. Further randomized controlled study is appropriate to confirm this possibility. Response heterogeneity amongst the subjects in terms of achieving resolution of certain symptoms rapidly suggests that future studies should explore additional dose amount and frequency options to determine if heterogeneous responsiveness can be overcome. Such response heterogeneity also presents an opportunity, in larger controlled studies, to understand more about the underlying pathologies of long COVID and how different mechanisms might be targeted by this and other approaches.

Conclusion

The hBM-MSC EV IP was safe following IV infusion of two doses in long COVID subjects. Improvements in quality of life, fatigue and pain metrics indicate that hBM-MSC EVs should be evaluated further as a potential novel and effective treatment for long COVID.

Author Contributions

Richard Bligh performed study design, patient recruitment, patient interaction, data acquisition/writing Robert Besancenez performed patient interaction and data acquisition.

Ethical Disclosure

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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Author Disclosures

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