Regenerative Engineering and Translational Medicine
https://doi.org/10.1007/s40883-025-00384-2

ORIGINAL RESEARCH

# Umbilical Cord-derived Mesenchymal Stem Cells (CLV-100) Infusion in Healthy Subjects: a 5-Year Follow-up Study on Safety and Immunomodulatory Effect

**Sze Piaw Chin[1,2,3] · Wan-Chiew Ng[2] · Lihui Tai[2] · Muzaida Aminah Mohd[2] · Kong Yong Then[2] · Soon Keng Cheong[3]**

Received: 8 January 2024 / Revised: 10 December 2024 / Accepted: 9 January 2025


## Abstract

**Purpose** Umbilical cord-derived mesenchymal stem cells (UCMSCs) have garnered significant attention in the field of regenerative medicine, specifically in inflammation-related diseases. Our team has previously demonstrated that a single infusion of allogeneic UCMSCs by Cytopeutics® (CLV-100), at both low dose (65 million cells) and high dose (130 million cells), in 11 healthy volunteers was safe up to 6 months with a dose-dependent immunomodulatory effect. In this present follow-up study, we investigated the medium-term safety and efficacy of intravenous administration of CLV-100 in the same healthy subjects.

**Methods** All 11 subjects were enrolled in this 5-year follow-up, and none dropped out (NMRR-13–1152-17400, 28/09/2016). Clinicians conducted consultations to assess overall health indicators. Blood samples and serum were collected for laboratory testing and biomarker analysis, respectively.

**Results** Five years after the CLV-100 infusion, all subjects remained healthy, with no reported side effects or major health conditions. Levels of cancer markers also remained within normal ranges. The anti-inflammatory effect of CLV-100 remained statistically significant in the high-dose group. Notably, major organ health parameters, including those subjects aged over 60 years, remained stable even after 5 years.

**Conclusion** The findings demonstrated the medium-term safety of a single CLV-100 infusion and persistent dose-dependent immunomodulatory effects. CLV-100 may also confer protection against age- and inflammation-related frailty.

**Lay Summary** The current follow-up study provides insight into the safety and immunomodulatory effects of a single infusion of allogeneic umbilical cord-derived mesenchymal stem cells (CLV-100) for up to 5 years in healthy subjects. The effects on the inflammatory cytokines CLV-100 were more pronounced in the high-dose group. In addition, other biomarker levels were comparable between the older and younger age groups. CLV-100 is therefore safe over the medium-term with sustained anti-inflammatory effect in a dose-dependent manner.

**Keywords** Umbilical cord-derived mesenchymal stem cells · Immunomodulation · Inflammation · Medium-term safety · Aging

## Introduction

Mesenchymal stem cells (MSCs) present a promising approach for treating inflammation-related diseases [1]. Among the frequently explored sources of MSCs are those derived from the umbilical cord (UCMSCs). UCMSCs are usually obtained from allogeneic sources due to their low immunogenicity, reducing the likelihood of triggering an immune response [2]. UCMSCs are well known for their good plasticity, immunomodulatory effects and ability to promote repair and regeneration in various tissue types. They are capable of reducing inflammation, thereby

✉ Sze Piaw Chin
chin.sze.piaw@gmail.com

1 CMH Specialist Hospital, 70200 Seremban, Negeri Sembilan, Malaysia

2 Cytopeutics Sdn. Bhd, Bio-X Centre, 63000 Cyberjaya, Selangor, Malaysia

3 M. Kandiah Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman, 43000 Kajang, Selangor, Malaysia

Published online: 28 January 2025

modulating the degree of injury and promoting effective healing or repair. This process mainly involves paracrine interactions with immune cells [3, 4]. Besides, UCMSCs are isolated from medical waste, involve non-invasive procedures, pose minimal ethical issues and are readily available, making them more attractive to researchers in translational medicine [5]. Particularly noteworthy is that UCMSCs obtained from neonatal umbilical cord exhibit greater activity and primitiveness than MSCs derived from bone marrow or adipose tissue [6], while being more stable than embryonic stem cells due to their tissue specificity [7].

Over the past few decades, comprehensive preclinical studies in mice and rat models have demonstrated the safety of UCMSC infusions at varying doses without detecting any tumourigenic risk [8–10]. In clinical trial settings, close follow-up was conducted in various diseases, such as severe COVID-19 (2 years), type II diabetes (3 years), and spinal cord injury (22.65 months), confirming that UCMSCs infusion is generally safe over the short term [11–13]. Some studies have monitored patients over longer periods to establish medium-term safety profiles in diseased patients, for example, up to 6 years in systemic lupus erythematosus among the Chinese population [14], 75 months in liver cirrhosis in the Chinese population [15] and 5 years in ischemic stroke in a South Korean study [16]. These results indicate that UCSMCs are potentially safe over medium-term follow-up. While this is encouraging, it is important to contribute further to this body of evidence, especially considering that UCMSCs derived and processed differently may not all be the same. Hence, this 5-year follow-up study using allogeneic UCMSCs specifically derived from the Malaysian population to evaluate medium-term safety is an important prerequisite before embarking on clinical trials for chronic medical conditions.

We have previously reported that a single infusion of allogeneic UCMSCs from Cytopeutics®, known as CLV-100, was safe in healthy subjects for up to 6 months with no apparent immediate or short-term reactions [17]. We further demonstrated that the immunomodulatory effects of CLV-100 were observed mainly in the high-dose group receiving 130 million cells [17]. This is likely due to the presence of a large number of proteins in the secretome of our UCMSCs, more than half of which are involved in immunomodulatory and anti-inflammatory mechanisms [18]. We intended to evaluate if the anti-inflammatory effects sustained up to 5 years by measuring the same cytokines during follow-up. Therefore, the current 5-year follow-up study aims to investigate the medium-term safety profile and immunomodulatory effects. This study represents the longest safety follow-up period of UCMSCs treatment in healthy subjects conducted in Malaysia to date.

## Method

### Study Design

In our previous phase I safety study of allogeneic infusion of UCMSCs from Cytopeutics® (CLV-100) in healthy volunteers (NMRR-13–1152–17400), a total of 11 subjects were enrolled between May 2017 and January 2018. The recruited subjects were divided into low-dose (LD) or high-dose (HD) groups and received a single infusion of CLV-100 of 65 million cells ($n=5$) or 130 million cells ($n=6$), respectively. These doses corresponded approximately with 1 million or 2 million cells per kilogram body weight ($kg^{-1}$ BW). Follow-up visits were conducted on days 2, 30, 90, and 180, with the results previously reported [17]. Due to the coronavirus disease 2019 (COVID-19) pandemic in Malaysia, follow-up between 2019 and 2022 was not possible. Despite this, all subjects maintained contact with the clinic annually. Accordingly, a 5-year follow-up visit (March and April 2023) was conducted at the outpatient clinic of CMH Specialist Hospital (Seremban, Negeri Sembilan, Malaysia), and all subjects were invited to participate in the follow-up. Data were collected to evaluate the medium-term safety and immunomodulatory effect of CLV-100 infusion in the 11 healthy subjects. All subjects provided consent before participating in the follow-up study.

### Outcome Measures

The primary outcomes were to determine the medium-term safety (clinical and subclinical outcomes) of CLV-100 infusion, with a focus on death, hospitalisation, related adverse events and cancer. The secondary outcomes included immunomodulatory effects based on sub-clinical serum assessment of inflammatory cytokines.

### Clinical and Subclinical Procedures

In the follow-up session, clinicians assessed the subjects and gathered information on their medical history from the preceding 5 years. Approximately 20 mL of peripheral blood was collected from each subject. Several clinical and subclinical assessments were performed, namely a) anthropometry data, b) lung function tests, c) blood tests including renal function tests, liver function tests, full blood count, pro- and anti-inflammatory cytokines and growth factors.

To identify cytokines and growth factors, subject sera were obtained through centrifugation and subsequently stored at − 80 °C until the testing phase. Anti-inflammatory interleukin IL-1 receptor antagonist (IL-1Ra) and two pro-inflammatory cytokines namely IL-6 and tumour necrosis

factor-alpha (TNF-α) were selected. For growth factor quantification, transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) were determined. IL-1Ra, IL-6, TNF-α, VEGF, and HGF were quantified using multiplex array kits (R&D System, Minneapolis, MN, USA), while TGF-β was tested with ELISA kits (R&D System, USA). All tests were performed in triplicate following the manufacturer's instruction, except for TGF-β which was performed in duplicate.

### Statistical Analysis

The small number of recruited subjects was regarded as non-normally distributed data. Therefore, the Friedman test was performed to compare the blood test markers at different time points: baseline, 6-month and 5-year follow-up, and biomarkers quantification at two time points: baseline and 5-year follow-up in both LD and HD groups. Then, Wilcoxon signed rank test was performed to test the pairwise comparison. Meanwhile, Mann–Whitney test was conducted to compare between two groups. All data were presented as median ± interquartile range (IQR). Statistical significance was considered when $p < 0.05$. Data analysis was performed using IBM SPSS Statistic v23.0 software (SPSS, Inc., Armonk, NY, USA) and graphs were plotted using Prism v8.0 (GraphPad Software, Boston, MA, USA).

## Result

### Follow-up Characteristics of Subjects

All 11 healthy volunteers, comprising four men and seven women, agreed to participate in the follow-up study. Among them, five had previously received a low dose (LD) of CLV-100 (65 million cells) and six had received a high dose (HD) of CLV-100 (130 million cells) (Fig. 1). At this five-year follow-up, the median age of the subjects was 56 ± 20 years, with a body mass index (BMI) of 22.40 ± 5.15 kg/m$^2$ (Table 1). All subjects remained functionally independent, physically active and healthy, as indicated by anthropometric data (Table 1). Notably, only three subjects were slightly overweight, with a BMI > 25 kg/m$^2$, and two subjects had shifted from a normal BMI to slightly underweight.

### Primary Outcomes: Adverse Events and Biomarkers

There were no major health issues, major surgeries and record of hospitalisations reported in either the LD or HD groups. The medical histories of the patients are summarised in Table 2. Subject 6 was diagnosed with hypercholesterolaemia and commenced on a lipid-lowering medication, subject 7 underwent surgery for her pre-existing benign uterine

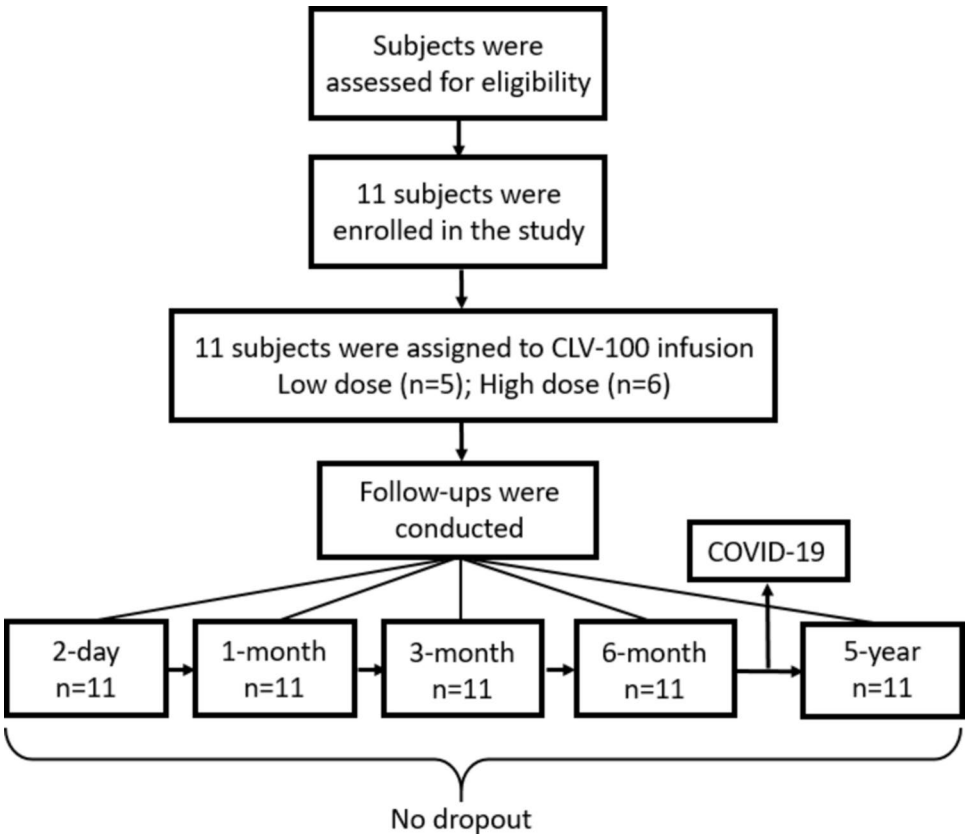


**Fig. 1** Flow chart outlines the process from subjects' recruitment to the 5-year follow-up

**Table 1** Anthropometry data of the low-dose (LD) and high-dose (HD) groups during the 5-year follow-up

| Subject | Dose | Gender | Age | Height (cm) | Weight (kg) | Baseline BMI (kg/m$^2$) | 5-year BMI (kg/m$^2$) |
|---|---|---|---|---|---|---|---|
| 1 | LD | F | 50 | 157.0 | 42.0 | 18.69 | 17.04 |
| 2 | LD | F | 82 | 157.0 | 45.0 | 19.50 | 18.26 |
| 3 | LD | F | 52 | 166.1 | 59.5 | 19.84 | 21.60 |
| 4 | LD | M | 46 | 165.0 | 54.0 | 20.56 | 19.83 |
| 5 | LD | M | 61 | 167.2 | 73.2 | 23.66 | 26.20 |
| 6 | HD | M | 82 | 169.0 | 70.0 | 24.85 | 24.51 |
| 7 | HD | F | 47 | 169.0 | 59.9 | 18.55 | 21.01 |
| 8 | HD | F | 70 | 154.0 | 61.0 | 24.87 | 25.72 |
| 9 | HD | F | 65 | 156.0 | 64.0 | 24.65 | 26.30 |
| 10 | HD | F | 56 | 169.0 | 60.0 | 22.40 | 21.01 |
| 11 | HD | M | 56 | 176.0 | 75.0 | 23.56 | 24.21 |
| Median ± IQR | | | 56.00 ± 20.00 | 166.10 ± 12.00 | 60.00 ± 16.00 | 21.60 ± 5.89 | 22.40 ± 5.15 |

**Table 2** Medical history of all subjects ($n = 11$)

| Subject | Medical history | |
|---|---|---|
| | Pre-existing | 5-year follow-up |
| 1 | Previous dilation and curettage (D&C)<br>Occasional dizziness and imbalance | None |
| 2 | Palpitations<br>Medication: Bisoprolol 2.5 mg | None |
| 3 | None | None |
| 4 | None | None |
| 5 | None | None |
| 6 | None | High cholesterol<br>Medication: started on atorvastatin 5 mg/day |
| 7 | Breast cyst, benign uterine fibroids, previous gallbladder removal, heartburn, bloatedness | Operation on pre-existing benign uterine fibroids in November 2021<br>Breast cyst, with no increase in size or number |
| 8 | None | None |
| 9 | Cervical spondylosis, left shoulder muscle tear | None |
| 10 | None | None |
| 11 | None | Cataract operation |

fibroids in November 2021, while subject 11 underwent an elective cataract operation. In short, no CLV-100 related adverse events were reported at any time during the 5-year follow-up period.

The laboratory test results at 5-year follow-up were within the normal reference range for all subjects (Table 3). Compared to baseline measurements, there were significant increase in haemoglobin, haematocrit (HCT) and mean corpuscular volume (MCV). There was also a significant elevation in erythrocyte sedimentation rate (ESR) at 5-year follow-up compared to 6 months. This increase is expected as ESR typically rises with age, and the values remained within the normal range (< 30 mm/h) for this cohort (median age 56.0 years).

Lipid indicators such as cholesterol, triglyceride levels, cholesterol/high-density lipoprotein (HDL) ratio, and diabetes screening markers such as glucose and glycated haemoglobin (HbA1c), remained unchanged and within normal ranges in all 11 subjects 5 years after CLV-100 infusion. Fasting serum insulin levels showed an increase when comparing between 6 months and 5 years but remained within the normal range (5–15 mIU/L).

Among the tumour markers, there were no significant increase from baseline for total prostate-specific antigen (PSA), cancer antigen (CA) 125, CA15.3, and alpha-fetoprotein (AFP) in blood tests; these markers remained within normal ranges. Serum CA19.9 and carcinoembryonic antigen (CEA) showed a significant increase at 5-year

**Table 3** Clinical assessments of all subjects ($n = 11$)

| Parameters | Baseline day 0 | Follow-up period | | *p*-value |
|---|---|---|---|---|
| | | 6 months | 5 years | |
| Full blood count | | | | |
| WBC ($\times 10^9$/L) | 6.6 ± 1.2 | 6.1 ± 1.5 | 6.3 ± 2.2 | 0.14 |
| Haemoglobin (g/dL) | 13.1 ± 2.7[a] | 13.5 ± 2.8 | 13.6 ± 1.7[a] | **0.04** |
| HCT (%) | 40.0 ± 6.0[a] | 42.0 ± 7.0 | 43.0 ± 4.0[a] | **0.01** |
| MCV (fl) | 86.8 ± 5.0[a] | 87.0 ± 6.0[b] | 90.0 ± 5.0[ab] | **< 0.01** |
| Platelet ($\times 10^9$/L) | 279.0 ± 103.0 | 286.0 ± 101.0 | 293.0 ± 87.0 | 0.34 |
| ESR (mm/h) | 10.0 ± 31.0 | 12.0 ± 15.0[b] | 23.0 ± 20.0[b] | **0.03** |
| Lipid tests | | | | |
| Total cholesterol (mmol/L) | 5.3 ± 1.0 | 5.6 ± 2.3 | 5.1 ± 1.2 | 0.51 |
| Triglyceride (mmol/L) | 1.3 ± 0.6 | 1.3 ± 1.0 | 1.2 ± 0.3 | 0.76 |
| HDL (mmol/L) | 1.4 ± 0.4 | 1.4 ± 0.4 | 1.5 ± 0.6 | 0.53 |
| LDL (mmol/L) | 3.4 ± 1.2 | 3.9 ± 1.7 | 3.3 ± 1.3 | 0.76 |
| Cholesterol/HDL ratio | 4.2 ± 1.4 | 4.3 ± 1.7 | 3.7 ± 0.8 | 0.91 |
| Diabetes screening | | | | |
| Glucose (mmol/L) | 4.6 ± 0.4 | 4.7 ± 0.4 | 4.9 ± 0.3 | 0.70 |
| HbA1c (%) | 5.5 ± 0.4 | 5.7 ± 0.6 | 5.7 ± 0.4 | 0.54 |
| Insulin (mIU/L) | 4.5 ± 3.6 | 3.7 ± 0.7[b] | 6.1 ± 4.6[b] | **0.04** |
| Allergy test | | | | |
| hs-CRP (mg/L) | 0.7 ± 1.0 | 1.0 ± 1.8 | 0.5 ± 1.4 | 0.15 |
| IgE (IU/mL) | 14.4 ± 46.5 | 16.9 ± 49.4 | 17.3 ± 22.7 | 1.00 |
| Tumour marker | | | | |
| Total PSA[#] (ng/mL) | 1.9 ± 1.4 | 1.3 ± 1.5 | 1.6 ± 2.7 | 0.28 |
| CA125[##] (U/mL) | 7.9 ± 3.8 | 8.0 ± 6.3 | 7.0 ± 3.4 | 0.28 |
| CA15.3[##] (U/mL) | 7.9 ± 8.6 | 9.5 ± 7.3 | 9.3 ± 8.0 | 0.07 |
| CEA (ng/mL) | 0.7 ± 0.8[a] | 0.7 ± 0.8[b] | 1.6 ± 1.4[ab] | **0.01** |
| CA19.9 (U/mL) | 17.6 ± 17.2 | 16.3 ± 16.7[b] | 23.0 ± 25.1[b] | **0.01** |
| AFP (ng/mL) | 2.2 ± 4.7 | 1.6 ± 3.3 | 3.40 ± 3.0 | 0.07 |
| Liver function tests | | | | |
| Total protein (g/L) | 74.0 ± 5.0 | 73.0 ± 5.0 | 72.0 ± 7.0 | 0.18 |
| Bilirubin (μmol/L) | 12.4 ± 5.0 | 12.0 ± 6.0 | 11.0 ± 5.0 | 0.18 |
| Alkaline phosphate (U/L) | 61.0 ± 36.0 | 63.0 ± 19.0 | 68.0 ± 35.0 | 0.39 |
| GGT (U/L) | 21.0 ± 10.0 | 21.0 ± 13.0 | 19.0 ± 14.0 | 0.16 |
| AST (IU/L) | 20.0 ± 6.0 | 22.0 ± 7.0 | 20.0 ± 7.0 | 0.15 |
| ALT (IU/L) | 17.0 ± 12.0 | 18.0 ± 4.0 | 17.0 ± 15.0 | 0.64 |
| Albumin (g/L) | 43.0 ± 5.4 | 43.0 ± 3.0 | 44.0 ± 5.0 | 0.82 |
| Globulin (g/L) | 31.0 ± 3.0 | 29.0 ± 4.0 | 29.0 ± 4.0 | 0.16 |
| A/G ratio | 1.4 ± 0.4 | 1.5 ± 0.2 | 1.6 ± 0.2 | 0.39 |
| Lung function tests | | | | |
| FEV1 (L) | 2.3 ± 0.9[a] | 2.3 ± 0.4 | 1.89 ± 1.1[a] | **0.01** |
| FVC (L) | 2.9 ± 0.8[a] | 2.8 ± 0.9 | 2.35 ± 1.4[a] | **0.02** |
| FEV1/FVC (%) | 87.0 ± 13.6 | 82.9 ± 14.0 | 72.64 ± 13.7 | 0.08 |
| Renal profile | | | | |
| Creatinine (μmol/L) | 65.0 ± 32.0 | 68.0 ± 26.0 | 70.0 ± 17.0 | 0.16 |
| eGFR (mL/min) | 60.0 ± 3.0 | > 60.0 | > 60.0 | 0.05 |
| Urea (mmol/L) | 4.2 ± 1.5 | 4.3 ± 1.9 | 4.6 ± 2.4 | 0.41 |
| Hormonal status | | | | |
| TSH (mU/L) | 1.5 ± 1.5 | 1.3 ± 1.1 | 1.7 ± 1.2 | 0.61 |
| Free T3 (pmol/L) | 5.0 ± 1.3 | 4.9 ± 1.4 | 5.1 ± 0.8 | 0.47 |
| Free T4 (pmol/L) | 16.9 ± 5.9 | 15.8 ± 3.8 | 15.4 ± 3.5 | 0.16 |

**Table 3** (continued)

| Parameters | Baseline day 0 | Follow-up period | | *p*-value |
|---|---|---|---|---|
| | | 6 months | 5 years | |
| FSH[##] (IU/L) | 9.1 ± 32.6 | 6.2 ± 63.0 | 61.4 ± 43.4 | 0.16 |
| LH[##] (IU/L) | 5.8 ± 10.2 | 3.8 ± 26.9 | 28.4 ± 25.4 | 0.37 |
| IGF-1(ng/mL) | 169.5 ± 111.3[a] | 133.5 ± 98.2[b] | 105.0 ± 48.3[ab] | **0.01** |
| DHEAS (µmol/L) | 2.5 ± 3.8 | 1.85 ± 2.6 | 1.90 ± 2.3 | 0.79 |
| Estradiol[##] (pg/mL) | 22.2 ± 128.1 | 87.4 ± 141.2 | 16.3 ± 90.0 | 0.74 |
| Progesterone[##] (ng/mL) | 0.2 ± 3.4 | 0.2 ± 17.8 | 0.3 ± 9.2 | 0.10 |
| Testosterone[#] | 3.8 ± 2.1 | 4.1 ± 2.1 | 4.0 ± 0.4 | 1.00 |

Friedman test was conducted, and the *p*-value was indicated in the column. Post hoc testing was conducted using the Wilcoxon signed-rank test. [a]Significant difference between baseline and 5 years. [b]Significant difference between 6 months and 5 years; [#]Only for male subjects ($n = 4$); [##] Only for female subjects ($n = 7$). *WBC*, white blood cells; *HCT*, haematocrit; *MCV*, mean corpuscular volume; *ESR*, erythrocyte sedimentation rate; *HDL*, high-density lipoprotein; *LDL*, low-density lipoprotein; *HbA1c*, glycated haemoglobin; *hs-CRP*, high-sensitivity C-reactive protein; *IgE*, immunoglobulin E; *IGF-1*, insulin growth factor-1; *PSA*, prostate-specific antigen, *AFP*, alpha fetoprotein; *GGT*, gamma-glutamyl transferase; *AST*, aspartate transferase; *ALT*, alanine transaminase; *A/G ratio*, albumin/globulin ratio; *FEV1*, forced expiratory volume; *FVC*, forced vital capacity; *eGFR*, estimated glomerular filtration rate; *TSH*, thyroid-stimulating hormone; *FSH*, follicle-stimulating hormone; *LH*, luteinizing hormone; *DHEAS*, dehydroepiandrosterone sulfate. Boldfaced entries are to emphasized the significant changes ($p < 0.05$) for the parameters

mark compared to values at 6 months, but these levels also remained well within the normal range.

Several important markers representing liver function, namely total protein, bilirubin, alkaline phosphate, gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), and alanine transaminase (ALT), as well as markers for renal function such as creatinine, estimated glomerular filtration rate (eGFR) and urea, were all within normal ranges and showed no significant differences from baseline values.

The lung function tests, represented by forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC), decreased significantly when comparing baseline measurements to those at 5 years. However, the FEV1/FVC ratio remained stable. Other indicators of allergy and inflammation, such as high-sensitivity C reactive protein (hs-CRP) and immunoglobulin E (IgE) levels, remained low and within the normal range for both groups.

Despite some subjects being over 60 years old, hormone levels such as dehydroepiandrosterone sulfate (DHEAS), estradiol, progesterone and testosterone remained comparable to baseline levels. However, insulin-like growth factor-1 (IGF-1) significantly decreased over the 5-year period.

## Secondary Outcomes: Inflammatory Cytokines and Growth Factors

The same inflammatory markers and growth factors were measured to understand the effect of CLV-100 on biomarkers at different time points. All data from the LD and HD groups were computed, and baseline and 5-year values were plotted (Fig. 2). Yet, within the LD group, data from two samples at the baseline time point became unusable due to sample degradation. Data showed that the pro-inflammatory TNF-α (89.6 ± 48.0 pg/mL vs < 7.4 pg/mL; $p < 0.01$) and IL-6 (14.0 ± 7.7 pg/mL vs 1.52 ± 5.7 pg/mL; $p < 0.01$) decreased significantly, while the anti-inflammatory IL-1Ra (477.3 ± 186.5 pg/mL vs 651.7 ± 862.2 pg/mL; $p = 0.021$) increased significantly from baseline to 5 years. Note

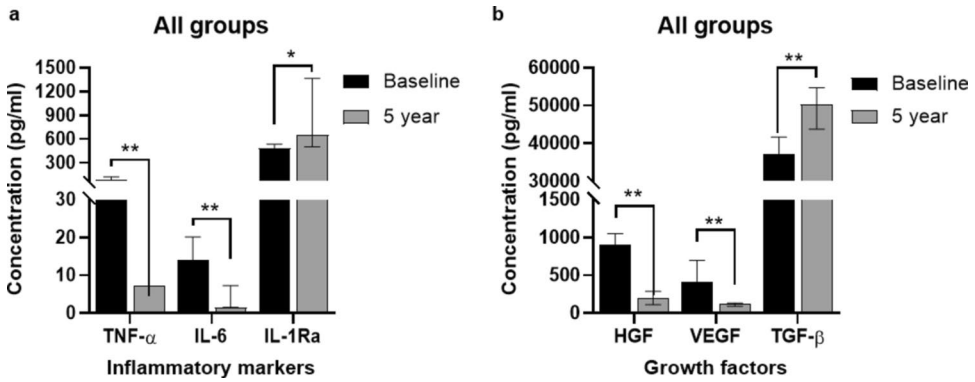


**Fig. 2** The levels of inflammatory markers (**a**) and growth factors (**b**) in all ($n = 11$) groups comparing between baseline and 5-year follow-up. Wilcoxon signed-rank test was conducted to compare the two time points. *$p < 0.05$, **$p < 0.01$

that for subjects with values falling outside the standard curve, the lowest detection concentrations were reported, which are 7.4 pg/mL for TNF-α and 1.5 pg/mL for IL-6. On the other hand, levels of HGF (900.0 ± 489.2 pg/mL vs 195.6 ± 176.0 pg/mL; $p < 0.01$) and VEGF (420.9 ± 466.3 pg/mL vs 119.9 ± 50.5 pg/mL; $p < 0.01$) reduced, whereas TGF-β (37,296.6 ± 9941.0 pg/mL vs 50,280.0 ± 10,940.0 pg/mL; $p < 0.01$) increased during the 5-year follow-up.

When the biomarkers of the subjects were analysed separately for LD and HD groups, as shown in Figs. 3 and 4, significant differences were observed only in the HD groups. In the HD groups, the level of IL-1Ra increased after CLV-100 infusion and remained elevated at the 5-year follow-up (478.3 ± 196.5 pg/mL vs 1248.9 ± 796.7 pg/mL; $p = 0.028$). High levels of IL-1Ra were concordant with low levels of IL-6 (14.0 ± 5.2 pg/mL vs 3.3 ± 6.6 pg/mL; $p = 0.028$) and TNF-α (85.9 ± 43.0 pg/mL vs < 7.4 ± 2.12 pg/mL; $p = 0.028$) (Fig. 3a). Additionally, changes in serum growth factors were monitored. In the HD group, VEGF (440.7 ± 588.2 pg/mL vs 126.0 ± 97.5 pg/mL; $p = 0.028$) and HGF (938.3 ± 640.8 pg/mL vs 268.5 ± 145.2 pg/mL; $p = 0.028$) were significantly reduced, while TGF-β levels significantly increased at 5-year follow-up compared to baseline values (37,496.6 ± 8331.8 pg/mL vs 52,380.0 ± 12,022.5 pg/mL; $p = 0.028$) (Fig. 3b). In contrast, none of these inflammatory biomarkers and growth factors in the LD group showed statistically significant differences between baseline and the 5-year follow-up (Fig. 4).

Indeed, when comparing the biomarkers between the HD and LD groups, IL-1Ra (1248.9 ± 796.7 pg/mL vs 501.1 ± 233.0 pg/mL, $p = 0.028$) and HGF (268.5 ± 145.2 pg/mL vs 123.2 ± 59.9 pg/mL, $p = 0.045$) showed a significantly higher value in the HD group compared to the LD group at the 5-year follow-up (Fig. 5).

Age plays a vital role in influencing the level of biomarkers. Further subgroup analyses were performed to investigate the impact of CLV-100 infusion in different age groups, namely the young group (< 60 years old) and the older group (> 60 years old), categorised according to the Malaysian government's classification of elderly/senior citizens [19].

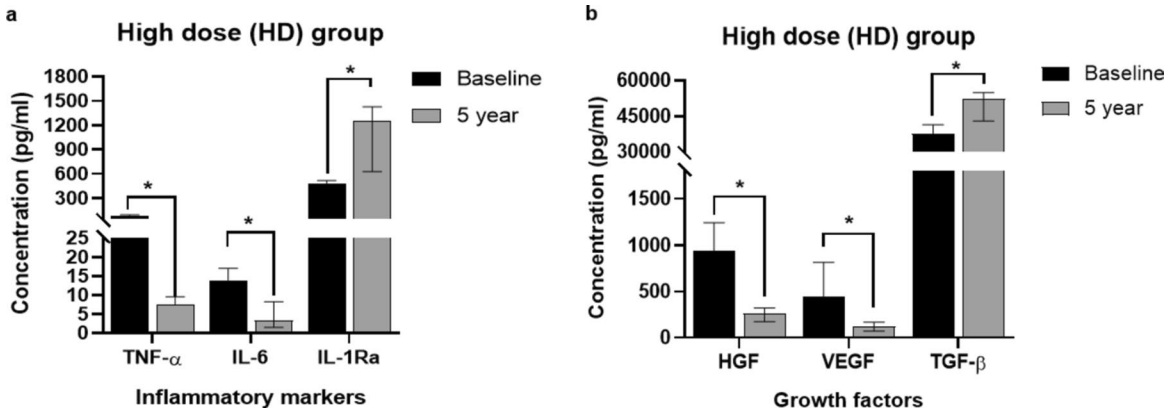


**Fig. 3** The levels of inflammatory markers (**a**) and growth factors (**b**) in the high-dose (HD; $n = 6$) group comparing between baseline and 5-year follow-up. Wilcoxon signed-rank test was conducted to compare the two time points. *$p < 0.05$

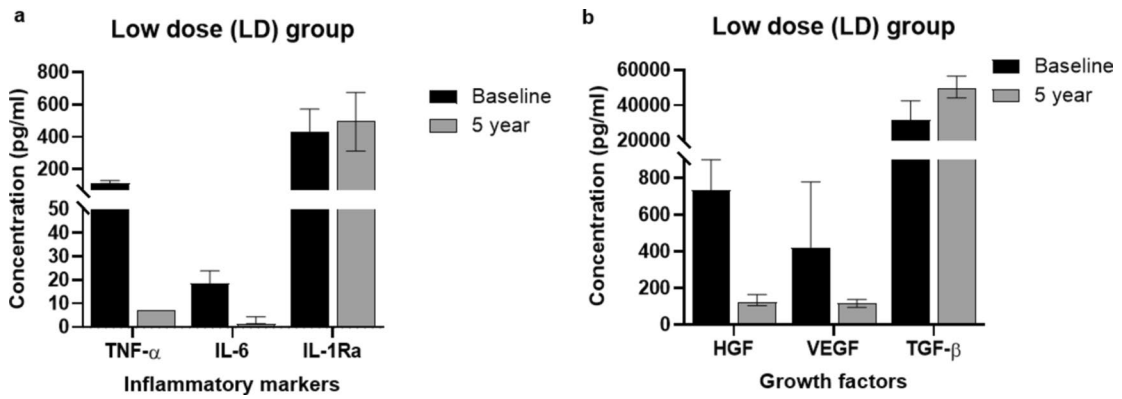


**Fig. 4** The levels of inflammatory markers (**a**) and growth factors (**b**) in the low-dose (LD; $n = 3$ in baseline, $n = 5$ in 5 years) group comparing between baseline and 5-year follow-up data. Wilcoxon signed-rank test was conducted to compare between two time points

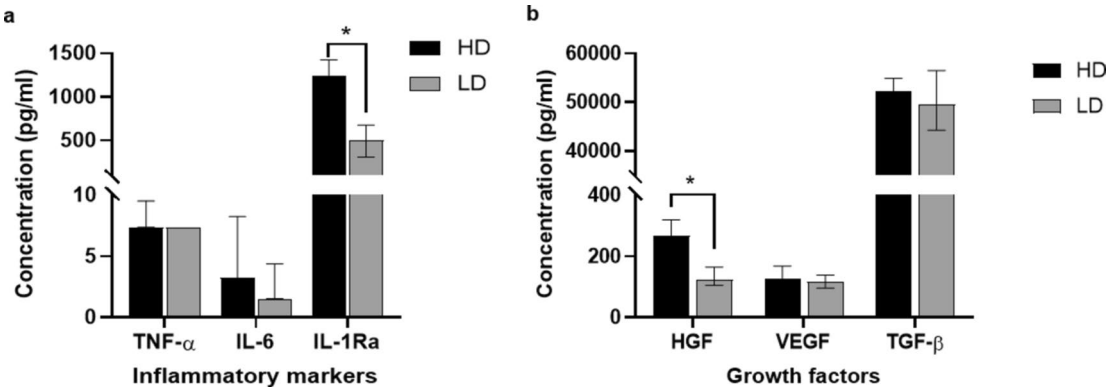


**Fig. 5** The levels of inflammatory markers (**a**) and growth factors (**b**) in the low-dose (LD; $n=5$) and high-dose (HD; $n=6$) groups during the 5-year follow-up. Mann–Whitney test was conducted to compare significant differences between LD and HD groups. $*p<0.05$

During the 5-year follow-up, six subjects were younger than 60 years old, while five subjects were 60 years old or older. Both the LD and the HD groups consisted of three young subjects; the LD group included two older subjects, and the HD group had three older subjects. It was observed that there was no significant difference in biomarkers between younger and older subjects 5 years after CLV-100 infusion (Fig. 6).

## Discussion

### CLV-100 Infusion is Safe in Medium-term

Our previous study demonstrated the safety and beneficial immunomodulatory and anti-inflammatory effects of both 65 million cells and 130 million cells of CLV-100 infusions in healthy subjects for up to 6 months [17]. In the current study, the medium-term safety and potential efficacy of CLV-100 infusion were evaluated over a 5-year follow-up period. There were no complications, hypersensitivity or adverse events reported. Based on the assessments, clinical parameters including full blood count, lipid tests, allergy tests, liver function tests, lung function tests, renal profile, and hormonal status remained within normal ranges. These results indicate that administration of CLV-100 does not lead to medium-term toxicity in major organs such as the lungs, liver and kidneys. Importantly, none of the subjects were diagnosed with cancer during the 5-year follow-up period.

Over the past 20 years, UCMSC therapy has been widely regarded as safe and well-tolerated in clinical studies. However, concerns persist regarding potential long-term side effects, including the risk of tumour formation. Circulating tumour markers are common screening tools for early detection of cancers [20]. In our 5-year follow-up investigation, there was no indication of underlying cancer in any of our patients, as indicated by clinical examinations and serum tumour marker levels, including total PSA, CA125, CA15.3, and AFP, which remained consistent with their respective baseline values. This outcome aligns with findings summarised by Xie et al. (2020), indicating that UCMSCs do not promote tumour formation but instead exhibit anti-tumour

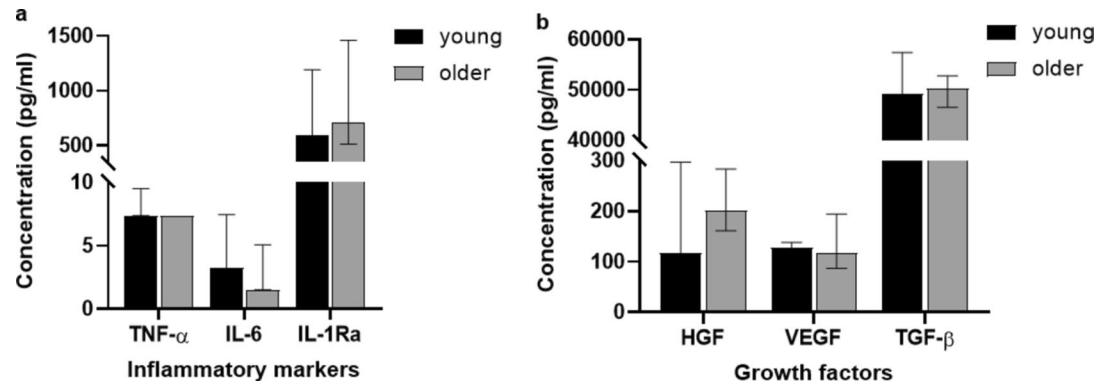


**Fig. 6** The levels of inflammatory markers (**a**) and growth factors (**b**) in the young (<60 years old; $n=6$) and older subjects (>60 years old; $n=5$) during the 5-year follow-up. Mann–Whitney test was conducted to compare significant difference between young and older subjects

properties (1). Although CA19.9 values in the HD group increased significantly at 5 years compared to 6 months, these values still remained within the normal range (< 37 U/mL) as defined by Ballehaninna and Chamberlain [21]. Similarly, CEA, an important protein involved in endothelial cell adhesion, proliferation, and migration, showed an increase in the LD group at 5 years compared with baseline and 6 months but remained within the normal range (< 3.0 µg/L) [22]. It is worth noting that CEA levels can be influenced by the age of subjects, with healthy older subjects typically exhibiting higher values than younger subjects [23]. In general, all subjects appeared healthy with no signs or symptoms suggestive of tumour development five years post-CLV-100 infusion.

In summary, clinician consultations, examinations, overall blood test reports, and biomarker testing consistently indicated the continued good health of all subjects. These findings are consistent with previous studies demonstrating the safety of UCMSCs treatment in patients with severe COVID-19 (2 years), type II diabetes (3 years), and systemic lupus erythematosus (6 years) [11, 12, 14]. However, it is important to acknowledge that diseased patients and healthy subjects may respond differently to UCMSCs infusion, especially to varying doses and infusion regimens, leading to diverse safety and efficacy outcomes. Moreover, factors such as donor variations, culture conditions, isolation techniques, cryopreservation methods, and thawing protocols can contribute to the heterogeneity of UCMSCs, affecting their stability, biological functions and properties [24]. Therefore, ongoing long-term monitoring of our participants following CLV-100 infusion is essential to comprehensively assess its safety and efficacy over time.

## CLV-100 Infusion Sustained the Immunomodulatory Effects for Up to 5 Years

UCMSCs are widely recognised for their immunomodulatory properties, which help maintain immune homeostasis in the host. They demonstrate pro-inflammatory effects in response to insufficient immune system activation and anti-inflammatory effects during excessive inflammatory activities [25]. In our study, CLV-100 infusion appeared to exert prolonged anti-inflammatory effects in healthy individuals, starting as early as day 2 after infusion, continuing through day seven, one month later [17], and persisting up to the 5-year follow-up, as indicated by high levels of IL-1Ra. IL-1Ra acts as an antagonist of IL-1, regulating and inhibiting the excessive systemic inflammatory effects of IL-1 [26]. Previous research has demonstrated that MSCs are effective in reducing damage and apoptosis of beta cells in diabetes mellitus *in vivo* models through the induction of IL-1Ra, which alleviates inflammation-induced injuries caused by IL-1 [27]. In contrast, reduced levels of IL-1Ra are associated with conditions such as cardiovascular disease, rheumatoid arthritis, and metabolic disorders [28, 29]. Interestingly, IL-1Ra levels differed significantly between the HD and LD groups (Fig. 5), suggesting that the higher dose of CLV-100 (130 million) exerts more pronounced anti-inflammatory properties. Nevertheless, it is important to note that elevated IL-1Ra levels have also been linked to an increase risk of developing metabolic syndrome. In healthy subjects aged 50–85 years, men typically exhibit IL-1Ra level of 133–137 pg/mL, while women exhibit levels of 117–144 pg/mL [30]. A threshold of over 408 pg/mL for IL-1Ra is suggested as indicative of prediabetes [29]. During our baseline screening and follow-ups, the healthy subjects showed high levels of IL-1Ra (> 400 pg/mL). However, no clinical manifestations such as abnormal glucose screening tests, lipid profiles or BMI were observed in the majority of subjects. There was an exception noted with increased insulin levels at the 5-year follow-up, but it remained within the normal range (3–15 mIU/L) [31]. Only one subject developed high cholesterol within this 5-year period.

Correspondingly, our study demonstrated a progressive decrease in IL-6 and TNF-α levels starting from day two after CLV-100 infusion, continuing through day 7, 1 month, 6 months [17], and remaining low at 5 years. These findings are consistent with previous publications indicating that UCMSC infusion reduced the expression of pro-inflammatory markers, including IL-1, IL-6, IL-8, TNF-α, and CRP in conditions such as arthritis (1) and sepsis [32]. The decline in pro-inflammatory markers can be attributed to the inverse relationship between IL-1Ra and IL-6/TNF-α, where an increase in IL-1Ra subsequently leads to decreased IL-6 and TNF-α levels. High levels of IL-6 and TNF-α are associated with an increased risk of coronary artery disease and metabolic syndrome. Hence, persistent low expression of these markers is beneficial for overall health [33, 34]. It is noteworthy that the decreased levels of IL-6 and TNF-α in our study did not indicate an immunosuppressed state in healthy subjects. In fact, their baseline IL-6 (LD: 18.6 ± 11.54 pg/mL; HD: 14.0 ± 5.2 pg/mL) and TNF-α (LD: 113.4 ± 15.3 pg/mL; HD: 85.9 ± 43.0 pg/mL) levels were higher than the reference range (TNF-α: 0.65–0.80 pg/mL and IL-6: 1.23–9.38 pg/mL) [35, 36], indicating a subclinical state of high inflammation before CLV-100 infusion, although the absence of clinical disease manifestations. This observation is consistent with the report by Tylutka et al. (2023), where high levels of IL-6 (41.0 ± 26.3 pg/mL) and TNF-α (72.3 ± 28.5 pg/mL) were detected in patients without metabolic syndrome, suggesting these markers as potential prediagnostic indicators for metabolic syndrome [37]. In our study, 5 years after CLV-100 infusion, the levels of IL-6 and TNF-α fell within the normal range. The normal reference range of each cytokine marker in healthy subjects is presented in Supplementary Table S1.

In this study, we also assessed the levels of HGF, VEGF, and TGF-β, the key growth factors and cytokines involved in inflammation-associated diseases. These factors regulate cellular proliferation, angiogenesis, tissue remodelling, and immune responses [38–46]. During the 5-year follow-up, the levels of HGF and VEGF in the subjects were lower, falling within the normal range for healthy subjects in this cohort (median age: 56.0 years) (HGF: 319–1475 pg/mL; VEGF: 46–983 pg/mL) [47, 48]. Under certain conditions, HGF can drive pro-inflammatory effects that contribute to the progression of diseases associated with persistent inflammation. HGF is recognised as an independent predictor of coronary heart disease, heart failure, stroke, and atherosclerosis progression [39, 49–52], and elevated serum levels of HGF have been observed in individuals with cardiovascular disease (CVD) risk factors such as smoking, obesity, hypertension, and diabetes [40, 52–55]. Therefore, the generally low HGF levels observed in both dose groups of healthy subjects may indicate the absence of underlying diseases linked to chronic inflammation. The VEGF levels were also low in all subjects in this study, which was consistent with the serum tumour marker levels aforementioned. While VEGF is crucial for angiogenesis and tissue repair after ischemic events, its dysregulation can contribute to chronic inflammation and tumour formation [56, 57]. Recent data demonstrated a strong link between chronic inflammation, angiogenesis, and the development of cancer. It is reported that the vascular changes associated with angiogenesis not only occur in cancer but also in other diseases in which chronic inflammation plays a major role, including cardiovascular disease, rheumatoid arthritis, diabetic retinopathy and asthma [42, 43]. Given that none of the subjects have cancer and other chronic inflammation related diseases, we are reassured that UCMSCs infusion does not induce pro-angiogenic effect in patients without ischemic conditions. The third growth factor assessed was TGF-β, a pleiotropic cytokine involved in both suppressive and inflammatory immune responses. Despite the significant increase in TGF-β levels compared to baseline, these values remained within the normal range. Okamoto et al. (2005) reported a mean TGF-β level of 40,300 ± 17,700 pg/mL in Japanese subjects aged 21 to 67 years old [58], further supporting the normalcy of our findings. Maintaining TGF-β level within normal range is critical as inadequate TGF-β signalling can result in dysregulation of naïve T cell proliferation, leading to an overactive immune system and impaired immune homeostasis, potentially contributing to autoimmune diseases, chronic inflammation, or excessive immune responses [44–46, 59].

Our results suggest that UCMSCs have the potential to modulate key pro-inflammatory cytokines and growth factors, though their effects may vary depending on factors such as the inflammatory environment, physiological conditions, and type of diseases. These findings highlight the complexity of UCMSC therapy and emphasise the need for further research to fully understand the mechanisms underlying their immunomodulatory and regenerative properties.

## Potential Effect of CLV-100 Infusion on Aging and Frailty

Another intriguing finding from our data is the potential of CLV-100 to mitigate the ageing process in elderly individuals. Ageing is characterised by the progressive deterioration of biological, physiological and psychological functions in the human body, often related to underlying inflammatory causes [60]. Ageing individuals typically exhibit high levels of pro-inflammatory cytokines such as TNF-α and IL-6 [61], which are associated with an increased risk of age-related diseases [62]. Additionally, declines in liver and kidney function, sex hormones, metabolic function, and increased prevalence of obesity are commonly observed with ageing [63, 64]. During the 5-year follow-up, it was observed that two subjects experienced a drop in BMI from normal to underweight, and most subjects showed a mild reduction in lung function, which remained within normal parameters. These occurrences are common manifestations of ageing, where physiological changes such as alterations in body composition, reductions in muscle mass index and bone density, are observed [63, 64]. Ageing also affects the respiratory system, leading to a loss of elasticity in both the airways and lung parenchyma, thinning of bones, and changes in the shape of the ribcage [65, 66], which consequently affect spirometric parameters. These changes correspond with elevated TGF-β levels observed at 5 years, which are involved in the regeneration of bone, cartilage, and skin during ageing [67].

Nevertheless, our study demonstrated that after 5 years, levels of the major organ parameters remained comparable, and the levels of pro-inflammatory cytokines reduced markedly compared to baseline in both dose groups, suggesting a potential protective outcome of CLV-100 against the effects of ageing. Specifically, serum testosterone levels were maintained in the subjects despite the expected decrease with age [68]. The IGF-1 levels declined to within the normal range (95–168 ng/mL) [69] at 5 years, indicating healthy ageing, as prolonged elevation of IGF-1 levels is generally undesirable due to their potential contribution to tumourigenesis [70]. Although ESR showed a significant elevation, the level remained within the normal range (< 30 mm/h) for individuals > 50 years old [71]. Importantly, all subjects, regardless of age, maintained a low inflammatory state (hs-CRP and ESR) and consistent levels of renal, liver, metabolic and hormonal markers over the five years. This effect may be partly attributed to the potential ability of UCMSCs to reduce local reactive

oxygen species and support regeneration through paracrine effects [72], which could help delay the cellular ageing process and maintain cellular homeostasis [73].

Previous reports have indicated that IL-1Ra levels decrease with age, rendering the elderly more susceptible to inflammation-related diseases [74]. Surprisingly, in this study, IL-1Ra levels did not decrease with age and were even higher than baseline in older subjects. None of the biomarkers in older subjects were significantly different from younger subjects (< 60 years old), suggesting a lack of age-related manifestation after 5 years. Taken together, our findings suggest that CLV-100 infusion may contribute to health maintenance in older subjects. However, it is important to note that as this study does not include a control arm, the observed effects are not definitively attributed solely to the CLV-100 infusion. The results may also be influenced by other factors, such as lifestyle choices, physical activity, nutrition, social engagement, and mental health strategies. It is possible that the subjects in this study are engaging in a combination of positive behaviours and lifestyle choices that might contribute to their overall physical and mental well-being.

## Limitation

Despite the promising outcomes of this 5-year follow-up study, several limitations warrant consideration. Firstly, due to limited serum availability, a multiplex array kit was utilised to quantify the levels of IL-6, TNF-α, IL-1Ra, VEGF, and HGF instead of the ELISA kits used in our previous study. Moreover, IL-10 and prostaglandin E2 were excluded from analysis because previous data on these biomarkers were extrapolated and inappropriate for direct comparison with the current study, and there was insufficient remaining blood serum for additional testing. This study also lacked a parallel placebo group as a control. The primary focus of our investigation was to confirm the medium-term safety and examine potential side effects of CLV-100 in healthy subjects. As such, the absence of a control group may limit the ability to make direct comparisons and draw definitive conclusions about the observed effects whether they are directly attributable to CLV-100 or due to other factors including changes in lifestyles, diet, psychological adaptation and other environmental factors. Lastly, no follow-up was conducted between 2019 and 2022 due to the COVID-19 pandemic and movement control orders in Malaysia, resulting in a missing gap in the data for the 1-year and 2-year follow-up periods. Considering these limitations, a larger, randomised controlled trial is believed to be necessary to confirm these findings and to better isolate the impact of CLV-100 infusion from other potential contributing factors.

## Conclusion

This 5-year follow-up study represents the first investigation to demonstrate the medium-term safety profile of CLV-100, a single administration of low or high doses of human umbilical cord-derived MSCs, in healthy subjects. Notably, no treatment-related adverse events, major health conditions or risk of tumour formation were reported throughout the study period, highlighting the safety of CLV-100 in this cohort. Significant immunomodulatory and anti-inflammatory effects of CLV-100 were observed, persisting for up to 5 years in both dose groups, with a more pronounced effect noted in the high-dose group. Interestingly, parameters of organ health remained stable after 5 years, even in subjects over 60 years of age, suggesting that the long-term effects of CLV-100 may have potential protective benefits against age-related health decline. This study represents a pioneering effort in exploring the possible applications of UCMSCs for frailty in clinical settings. Further research is warranted to fully explore the therapeutic potential of UCMSCs in aging and inflammation-related diseases.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s40883-025-00384-2.

**Acknowledgements** This work was supported by Cytopeutics Unconditional Research Grant. We want to thank CMH Specialist Hospital for providing the facilities to conduct this follow-up study. We are grateful to have Ms. Manimagelai from CMH Specialist Hospital in facilitating the project, and Dr. Wong Li Zhe and Mr. Excel Chok who assisted in this follow-up.

**Author Contribution** Sze Piaw Chin: conceptualisation, methodology, funding acquisition, supervision, validation, visualisation. Wan-Chiew Ng: data curation, formal analysis, visualisation, roles/writing—original draft. Lihui Tai: investigation, methodology, validation, project administration, resources, data curation. Muzaida Aminah Mohd: writing—review and editing. Kong Yong Then: writing—review and editing. Soon Keng Cheong: writing—review and editing.

**Data Availability** All data collected and generated in this study are included in the article and supplementary material or are available from the corresponding author upon reasonable request.

## Declarations

**Ethics Approval and Consent to Participate** This study obtained ethical approval from the National Medical Research Registry with code number NMRR-13–1152-17400, registered date 28/09/2016. All clinical investigations were conducted according to the Declaration of Helsinki.

**Consent for Publication** Written informed consent was obtained from the subjects before the research.

**Conflict of Interest** C.S.P advises Cytopeutics Sdn Bhd on regulatory, clinical, and research activities. C.S.K and T.K.Y sit on the Cytopeutics Sdn Bhd medical advisory board. T.L., N.W.C., and M.A.M. are the study's project coordinators.

## References

1. Xie Q, Liu R, Jiang J, Peng J, Yang C, Zhang W, Wang S, Song J. What is the impact of human umbilical cord mesenchymal stem cell transplantation on clinical treatment? Stem Cell Res Ther. 2020;11(519):1–13.
2. Shang Y, Guan H, Zhou F. Biological characteristics of umbilical cord mesenchymal stem cells and its therapeutic potential for hematological disorders. Front cell Dev Biol. 2021;9:570179.
3. Zhao T, Liang Q, Meng X, Duan P, Wang F, Li S, et al. Intravenous infusion of umbilical cord mesenchymal stem cells maintains and partially improves visual function in patients with advanced retinitis pigmentosa. Stem Cells Dev. 2020;29(16):1029–37.
4. Song N, Scholtemeijer M, Shah K. Mesenchymal stem cell immunomodulation: mechanisms and therapeutic potential. Trends Pharmacol Sci. 2020;41(9):653–64.
5. Mebarki M, Abadie C, Larghero J, Cras A. Human umbilical cord-derived mesenchymal stem/stromal cells : a promising candidate for the development of advanced therapy medicinal products. 2021;1–10.
6. Malgieri A, Kantzari E, Patrizi MP, Gambardella S. Bone marrow and umbilical cord blood human mesenchymal stem cells: state of the art. Int J Clin Exp Med. 2010;3(4):248–69.
7. Tesarova L, Jaresova K, Simara P, Koutna I. Umbilical cord-derived mesenchymal stem cells are able to use bFGF treatment and represent a superb tool for immunosuppressive clinical applications. Int J Mol Sci. 2020;21:5366.
8. Yun J-W, Ahn JH, Kwon E, Kim S-H, Kim H, Jang J-J, et al. Human umbilical cord-derived mesenchymal stem cells in acute liver injury: hepatoprotective efficacy, subchronic toxicity, tumorigenicity, and biodistribution. Regul Toxicol Pharmacol. 2016;81:437–47.
9. Chen G, Yue A, Ruan Z, Yin Y, Wang R, Ren Y, et al. Human umbilical cord-derived mesenchymal stem cells do not undergo malignant transformation during long-term culturing in serum-free medium. PLoS One. 2014;9(6):e98565.
10. Chin S-P, Saffery NS, Then K-Y, Cheong S-K. Preclinical assessments of safety and tumorigenicity of very high doses of allogeneic human umbilical cord mesenchymal stem cells. In Vitro Cell Dev Biol Anim. 2024.
11. Hu J, Wang Y, Gong H, Yu C, Guo C, Wang F, et al. Long term effect and safety of Wharton's jelly-derived mesenchymal stem cells on type 2 diabetes. Exp Ther Med. 2016;12:1857–66.
12. Li T-T, Zhang B, Fang H, Shi M, Yao W-Q, Li Y, et al. Human mesenchymal stem cell therapy in severe COVID-19 patients: 2-year follow-up results of a randomized, double-blind, placebo-controlled trial. EBioMedicine. 2023;92:104600.
13. Awidi A, Al SA, El AN, Alqudah M, Jamali F, Nazer F, et al. Safety and potential efficacy of expanded mesenchymal stromal cells of bone marrow and umbilical cord origins in patients with chronic spinal cord injuries: a phase I/II study. Cytotherapy. 2024;26:825–31.
14. Wang D, Niu L, Feng X, Yuan X, Zhao S, Zhang H, et al. Long-term safety of umbilical cord mesenchymal stem cells transplantation for systemic lupus erythematosus: a 6-year follow-up study. Clin Exp Med. 2017;17(3):333–40.
15. Shi M, Li Y-Y, Xu R-N, Meng F-P, Yu S-J, Fu J-L, et al. Mesenchymal stem cell therapy in decompensated liver cirrhosis: a long-term follow-up analysis of the randomized controlled clinical trial. Hepatol Int. 2021;15(6):1431–41.
16. Lee JS, Hong JM, Moon GJ, Lee PH, Ahn YH, Bang OY, et al. A long-term follow-up study of intravenous autologous mesenchymal stem cell transplantation in patients with ischemic stroke. Stem Cells. 2010;28(6):1099–106.
17. Chin S-P, Mohd-Shahrizal M-Y, Liyana M-Z, Then KY, Cheong SK. High dose of intravenous allogeneic umbilical cord-derived mesenchymal stem cells (CLV-100) infusion displays better immunomodulatory effect among healthy volunteers: a phase 1 clinical study. Stem Cells Int. 2020;2020:8877003.
18. Tai L, Saffery NS, Chin SP, Cheong SK. Secretome profile of TNF-α-induced human umbilical cord mesenchymal stem cells unveils biological processes relevant to skin wound healing. Regen Med. 2023;18(11):839–56.
19. Public Service Delivery and Local Government. In: The Elderly/Senior Citizens. My Government. 2021. https://www.malaysia.gov.my/portal/content/27752. Accessed 25 Oct 2023.
20. Nagpal M, Singh S, Singh P, Chauhan P, Zaidi MA. Tumor markers: a diagnostic tool. Natl J Maxillofac Surg. 2016;7(1):17–20.
21. Ballehaninna UK, Chamberlain RS. Serum CA 19–9 as a biomarker for pancreatic cancer—a comprehensive review. Indian J Surg Oncol. 2011;2(2):88–100.
22. Kankanala VL, Zubair M, Mukkamalla SKR. Carcinoembryonic antigen. [Updated 2024 Dec 11]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025. Available from: https://www.ncbi.nlm.nih.gov/books/NBK578172/
23. Touitou Y, Proust J, Klinger E, Nakache JP, Huard D, Sachet A. Cumulative effects of age and pathology on plasma carcinoembryonic antigen in an unselected elderly population. Eur J Cancer Clin Oncol. 1984;20(3):369–74.
24. Li J, Wu Z, Zhao L, Liu Y, Su Y, Gong X, et al. The heterogeneity of mesenchymal stem cells: an important issue to be addressed in cell therapy. Stem Cell Res Ther. 2023;14(1):1–14.
25. Jiang W, Xu J. Immune modulation by mesenchymal stem cells. Cell Prolif. 2020;53(1):e12712.
26. Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. Immunol Rev. 2018;281(1):8–27.
27. Volarevic V, Al-Qahtani A, Arsenijevic N, Pajovic S, Lukic ML. Interleukin-1 receptor antagonist (IL-1Ra) and IL-1Ra producing mesenchymal stem cells as modulators of diabetogenesis. Autoimmunity. 2010;43(4):255–63.
28. Almeida-Santiago C, Quevedo-Abeledo JC, Hernández-Hernández V, de Vera-González A, Gonzalez-Delgado A, González-Gay MÁ, et al. Interleukin 1 receptor antagonist relation to cardiovascular disease risk in patients with rheumatoid arthritis. Sci Rep. 2022;12(1):13698.
29. Luotola K. IL-1 receptor antagonist (IL-1Ra) levels and management of metabolic disorders. Nutrients. 2022;14(16):3422.
30. Ferrucci L, Corsi A, Lauretani F, Bandinelli S, Bartali B, Taub DD, et al. The origins of age-related proinflammatory state. Blood. 2005;105(6):2294–9.
31. Wilcox G. Insulin and insulin resistance. Clin Biochem Rev. 2005;26(2):19–39.
32. He X, Ai S, Guo W, Yang Y, Wang Z, Jiang D, et al. Umbilical cord-derived mesenchymal stem (stromal) cells for treatment of severe sepsis: a phase 1 clinical trial. Transl Res [Internet]. 2018. https://doi.org/10.1016/j.trsl.2018.04.006.
33. Kazemian S, Ahmadi R, Rafiei A, Azadegan-Dehkordi F, Khaledifar A, Abdollahpour-Alitappeh M, et al. The serum levels of IL-36

in patients with coronary artery disease and their correlation with the serum levels of IL-32, IL-6, TNF-α, and oxidative stress. Int Arch Allergy Immunol. 2022;183(10):1137–45.
34. Mohammadi M, Gozashti MH, Aghadavood M, Mehdizadeh MR, Hayatbakhsh MM. Clinical significance of serum IL-6 and TNF-α levels in patients with metabolic syndrome. Reports Biochem Mol Biol. 2017;6(1):74–9.
35. Wyczalkowska-Tomasik A, Czarkowska-Paczek B, Zielenkiewicz M, Paczek L. Inflammatory markers change with age, but do not fall beyond reported normal ranges. Arch Immunol Ther Exp (Warsz). 2016;64(3):249–54.
36. Scalzo P, Kümmer A, Cardoso F, Teixeira AL. Serum levels of interleukin-6 are elevated in patients with Parkinson's disease and correlate with physical performance. Neurosci Lett. 2010;468(1):56–8.
37. Tylutka A, Morawin B, Walas Ł, Michałek M, Gwara A, Zembron-Lacny A. Assessment of metabolic syndrome predictors in relation to inflammation and visceral fat tissue in older adults. Sci Rep. 2023;13(1):89.
38. Osibogun O, Ogunmoroti O, Ferraro RA, Ndumele CE, Burke GL, Larson NB, et al. Favorable cardiovascular health is associated with lower hepatocyte growth factor levels in the multi-ethnic study of atherosclerosis. Front Cardiovasc Med. 2021;8(January):1–7.
39. Bell EJ, Larson NB, Decker PA, Pankow JS, Tsai MY, Hanson NQ, et al. Hepatocyte growth factor is positively associated with risk of stroke: the MESA (Multi-Ethnic Study of Atherosclerosis). Stroke. 2016;47(11):2689–94.
40. Bancks MP, Bielinski SJ, Decker PA, Hanson NQ, Larson NB, Sicotte H, et al. Circulating level of hepatocyte growth factor predicts incidence of type 2 diabetes mellitus: the Multi-Ethnic Study of Atherosclerosis (MESA). Metabolism. 2016;65(3):64–72.
41. Guadagni F, Ferroni P, Palmirotta R, Portarena I, Formica V, Roselli M. TNF/VEGF cross-talk in chronic inflammation-related cancer initiation and progression: an early target in anticancer therapeutic strategy. In Vivo (Brooklyn). 2007;21(2):147–62.
42. Angelo LS, Kurzrock R. Vascular endothelial growth factor and its relationship to inflammatory mediators. Clin Cancer Res. 2007;13(10):2825–30.
43. Aguilar-Cazares D, Chavez-Dominguez R, Carlos-Reyes A, Lopez-Camarillo C, Hernadez de la Cruz ON, Lopez-Gonzalez JS. Contribution of angiogenesis to inflammation and cancer. Front Oncol. 2019;9(December):1–10.
44. Deng Z, Fan T, Xiao C, Tian H, Zheng Y, Li C, et al. TGF-β signaling in health, disease, and therapeutics. Signal Transduct Target Ther [Internet]. 2024;9(1). https://doi.org/10.1038/s41392-024-01764-w.
45. Sanjabi S, Oh SA, Li MO. Regulation of the immune response by TGF-β: from conception to autoimmunity and infection. Cold Spring Harb Perspect Biol. 2017;9(6):1–33.
46. Yoshimura A, Wakabayashi Y, Mori T. Cellular and molecular basis for the regulation of inflammation by TGF-β. J Biochem. 2010;147(6):781–92.
47. Malatino LS, Cataliotti A, Benedetto FA, Stancanelli B, Bellanuova I, Belluardo P, et al. Hepatocyte growth factor and left ventricular geometry in end-stage renal disease. Hypertension. 2003;41(1):88–92.
48. Werther K, Christensen IJ, Nielsen HJ, Group and the DRCCS. Prognostic impact of matched preoperative plasma and serum VEGF in patients with primary colorectal carcinoma. Br J Cancer. 2002;86(3):417–23.
49. Decker PA, Larson NB, Bell EJ, Pankow JS, Hanson NQ, Wassel CL, et al. Increased hepatocyte growth factor levels over 2 years are associated with coronary heart disease: the Multi-Ethnic Study of Atherosclerosis (MESA). Vol. 213, American heart journal. United States; 2019. p. 30–4.
50. Lamblin N, Susen S, Dagorn J, Mouquet F, Jude B, Van Belle E, et al. Prognostic significance of circulating levels of angiogenic cytokines in patients with congestive heart failure. Am Heart J. 2005;150(1):137–43.
51. Bell EJ, Decker PA, Tsai MY, Pankow JS, Hanson NQ, Wassel CL, et al. Hepatocyte growth factor is associated with progression of atherosclerosis: the Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis. 2018;272:162–7.
52. Yamamoto Y, Kohara K, Tabara Y, Miki T. Association between carotid arterial remodeling and plasma concentration of circulating hepatocyte growth factor. J Hypertens. 2001;19(11):1975–9.
53. Rehman J, Considine RV, Bovenkerk JE, Li J, Slavens CA, Jones RM, et al. Obesity is associated with increased levels of circulating hepatocyte growth factor. J Am Coll Cardiol. 2003;41(8):1408–13.
54. Nakamura T, Mizuno S. The discovery of hepatocyte growth factor (HGF) and its significance for cell biology, life sciences and clinical medicine. Proc Jpn Acad Ser B Phys Biol Sci. 2010;86(6):588–610.
55. Hiratsuka A, Adachi H, Fujiura Y, Yamagishi SI, Hirai Y, Enomoto M, et al. Strong association between serum hepatocyte growth factor and metabolic syndrome. J Clin Endocrinol Metab. 2005;90(5):2927–31.
56. Hicklin DJ, Ellis LM. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. J Clin Oncol. 2005;23(5):1011–27.
57. Holmes DIR, Zachary I. The vascular endothelial growth factor (VEGF) family: angiogenic factors in health and disease. Genome Biol. 2005;6(2):209.
58. Okamoto Y, Gotoh Y, Uemura O, Tanaka S, Ando T, Nishida M. Age-dependent decrease in serum transforming growth factor (TGF)-beta 1 in healthy Japanese individuals; population study of serum TGF-beta 1 level in Japanese. Dis Markers. 2005;21(2):71–4.
59. Sanjabi S, Zenewicz L, Kamanaka M, Flavell R. Anti- and pro-inflammatory roles of TGF-β, IL-10, and IL-22 in immunity and autoimmunity. Curr Opin Pharmacol. 2010;9(4):447–53.
60. Reale M. Inflammation in aging and age-related diseases. J Gerontol Geriatr Res. 2014;03(03):1–2.
61. Heinze-Milne SD, Banga S, Howlett SE. Frailty and cytokines in preclinical models: comparisons with humans. Mech Ageing Dev. 2022;206:111706.
62. Hartmann A, Hartmann C, Secci R, Hermann A, Fuellen G, Walter M. Ranking biomarkers of aging by citation profiling and effort scoring. Front Genet. 2021;12:686320.
63. JafariNasabian P, Inglis JE, Reilly W, Kelly OJ, Ilich JZ. Aging human body: changes in bone, muscle and body fat with consequent changes in nutrient intake. J Endocrinol. 2017;234(1):R37-51.
64. Amarya S, Singh K, Sabharwal M, Amarya S, Singh K, Sabharwal M. Ageing process and physiological changes. In: D'Onofrio G, Sancarlo D, Greco A. Gerontology. London: IntechOpen; 2018. pp. 3–24.
65. Park CH, Yi Y, Do JG, Lee YT, Yoon KJ. Relationship between skeletal muscle mass and lung function in Korean adults without clinically apparent lung disease. Med (United States). 2018;97(37):e12281.
66. Sanchis-Gimeno JA, Lois-Zlolniski S, María González-Ruiz J, Palancar CA, Torres-Tamayo N, García-Martínez D, et al. Association between ribs shape and pulmonary function in patients with osteogenesis imperfecta. J Adv Res. 2020;21:177–85.
67. Tirado-Rodriguez B, Ortega E, Segura-Medina P, Huerta-Yepez S. TGF- β: an important mediator of allergic disease and a molecule with dual activity in cancer development. J Immunol Res. 2014;2014:318481.
68. Chin K-Y, Soelaiman I-N, Mohamed IN, Ahmad F, Ramli ESM, Aminuddin A, et al. Sex hormones in Malay and Chinese men in

Malaysia: are there age and race differences? Clinics (Sao Paulo). 2013;68(2):159–66.
69. Stojanovic M, Popevic M, Pekic S, Doknic M, Miljic D, Medic-Stojanoska M, et al. Serum insulin-like growth factor-1 (IGF-1) age-specific reference values for healthy adult population of Serbia. Acta Endocrinol (Bucharest, Rom 2005). 2021;17(4):462–71.
70. Denduluri SK, Idowu O, Wang Z, Liao Z, Yan Z, Mohammed MK, et al. Insulin-like growth factor (IGF) signaling in tumorigenesis and the development of cancer drug resistance. Genes Dis. 2015;2(1):13–25.
71. Tishkowski K, Gupta V. Erythrocyte sedimentation rate. [updated 2023 Apr 23]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557485/
72. Li T, Zhou L, Fan M, Chen Z, Yan L, Lu H, et al. Human umbilical cord-derived mesenchymal stem cells ameliorate skin aging of nude mice through autophagy-mediated anti-senescent mechanism. Stem Cell Rev Rep. 2022;18(6):2088–103.
73. Liu M, He J, Zheng S, Zhang K, Ouyang Y, Zhang Y, et al. Human umbilical cord mesenchymal stem cells ameliorate acute liver failure by inhibiting apoptosis, inflammation and pyroptosis. Ann Transl Med. 2021;9(21):1615.
74. Zhao L, Wang L, Zhang X, Sun L, Sun D, Sun T. Reduced levels of interleukin-1 receptor antagonist act as a marker for pneumonia in the elderly. Mol Med Rep. 2014;10(2):959–64.