

**Clinical study of mesenchymal stem/stromal cell therapy for the treatment of frailty: a proposed experimental design for therapeutic and mechanistic investigation.**

Duc M. Hoang, PhD<sup>1</sup>, Kien T. Nguyen, MSc<sup>1</sup>, Van T. Hoang, PhD<sup>1</sup>, Lan T.M. Dao, PhD<sup>1</sup>, Hang T. Bui, MD<sup>2</sup>, Thanh T. K. Ho, MD, PhD<sup>3</sup>, Thuy T. P. Nguyen, MD<sup>4</sup>, Anh T.L. Ngo<sup>5</sup>, Hoa K. Nguyen MD, PhD<sup>6</sup>, Liem Nguyen Thanh MD, PhD<sup>1</sup>

<sup>1</sup>Vinmec Research Institute of Stem Cell and Gene Technology, Hanoi, Vietnam.

<sup>2</sup> Center of Regenerative Medicine and Cell Therapy, Vinmec Times City International Hospital, Hanoi, Vietnam.

<sup>3</sup> Hanoi Medical University, Hanoi, Vietnam.

<sup>4</sup> Dong Da General Hospital, Hanoi, Vietnam.

<sup>5</sup> Cell Therapy Department, Vinmec High-tech Center, Vinmec Healthcare System, Hanoi, Vietnam.

<sup>6</sup> Institute of Biomedical Engineering University of Toronto. Room 415, Rosebrugh Building, 164 College Street, Toronto, ON, M5S 3E2, Canada.

Correspondence to: Prof. Liem Nguyen Thanh (email: v.liemnt@vinmec.com), Vinmec Research Institute of Stem Cell and Gene Technology, Vinmec Health Care System, Hanoi, Vietnam.

© The Author(s) 2021. Published by Oxford University Press on behalf of The Gerontological Society of America.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<https://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact [journals.permissions@oup.com](mailto:journals.permissions@oup.com)

## Abstract

Frailty, a specific condition of increased vulnerability and reduced general health associated with aging in older people, is an emerging problem worldwide with major implications for clinical practice and public health. Recent preclinical and clinical studies have supported the safety of mesenchymal stem/stromal cells (MSCs) in the treatment of frailty. Comprehensive study is needed to assess the interrelationship between the condition of frailty and the effects of MSC-based therapy. This randomized controlled phase I/II trial aims to investigate the safety and potential therapeutic efficacy of the allogeneic administration of umbilical cord-derived MSCs (UC-MSCs) in combination with the standard treatment for frailty in Vietnam. Moreover, this study describes the rationales, study designs, methodologies and analytical strategies currently employed in stem cell research and clinical studies. The primary outcome measures will include the incidences of prespecified administration-associated adverse events (AEs) and serious adverse events (SAEs). The potential efficacy will be evaluated based on improvements in frailty conditions (including those determined through a physical examination, patient-reported outcomes, quality of life, immune markers of frailty, metabolism analysis, and cytokine markers from patient plasma). This clinical trial and stem cell analysis associated with patient sampling at different timepoints aim to identify and characterize the potential effects of UC-MSCs on improving frailty based on the stem cell quality, cytokine/growth factor secretion profiles of UC-MSCs, cellular senescence, and metabolic analysis of patient CD3+ cells providing fundamental knowledge for designing and implementing research strategies in future studies.

**Trial registration number:** NCT04919135

**Key words:** *umbilical cord, allogeneic cell therapy, stem cell phenotype, clinical trial.*

## Introduction

### *Frailty as a new frontier of medicine*

Frailty, a specific condition of increased vulnerability and reduced general health associated with aging in older people, is an emerging problem worldwide with major implications for clinical practice and public health (1). In countries with aging populations, including Vietnam, the prevalence of frailty is increasing rapidly (2). The estimated prevalence greatly varies between countries and ranges from 4 to 59% due to the lack of a nonstandardized definition or evaluation of frailty (3). In Vietnam, a study of 461 patients at the National Geriatric Hospital in Hanoi indicated that the prevalence of frailty according to the Reported Edmonton Frail Scale (REFS) was 31.9% (4). In 2001, a widely accepted clinical description of the disease was proposed by Fried and colleagues, and this description includes five major criteria: (1) unintentional weight loss, (2) weak grip strength, (3) low gait speed, (4) low physical activities, and (5) exhaustion (5). Based on these criteria, a patient who exhibits one to two symptoms is classified as prefrail, whereas a patient who presents at least three symptoms is diagnosed with frailty (Figure 1).

### *Rationale for Study Inception*

Frailty is strongly associated with oxidative stress and inflammation during the aging process, which includes the following major hallmarks: (1) instability of genomic materials, (2) reduction of telomerase activities and telomere attrition, (3) loss of proteostasis, (4) reduction of nutrient sensing, (5) metabolic malfunctions (including mitochondrial dysfunction), (6) cellular senescence, (7) stem cell depletion, and (8) alternation of cell-to-cell communication (Figure 1) (6). These aging hallmarks play a significant role in the development of other geriatric syndromes once frailty is established and progresses in its natural courses, which include cardiovascular disease, hypertension, arthritis, and diabetes

(7). Moreover, these aging-related features of frailty also alter endogenous stem cell regeneration and function, which in turn reduces the regenerative capacity of multiple organs and tissues. Toward this end, providing an exogenous stem cell population to replenish the stem cell pools and improve the regenerative ability has emerged as an alternative and promising approach for frailty (8).

To date, at least four clinical trials have been conducted to evaluate the safety and efficacy of MSC therapy in the treatment of frailty. Among them, two clinical trials (registered with the numbers NCT02982915 and NCT03169231) are ongoing multicenter, randomized, blinded, and placebo-controlled clinical studies using bone marrow-derived MSCs (9). Another trial, CRATUS (NCT02065245), was recently completed by Joshua M. Hare and colleagues (10). The results confirm that all participating patients tolerated the allogeneic administration of bone marrow-derived MSCs (BM-MSCs) well. In terms of efficacy, the trial indicated that 100 million cell doses were more effective than 200 million cell doses (11). In the CRATUS study, BM-MSCs were isolated and expanded from bone marrow aspirate from male or female donors between the ages of 20 and 45 with a comprehensive screening history and physical status (10). Once collected, the BM-MSCs were expanded in 20% fetal bovine serum (FBS)-supplemented media for 14 days and harvested at passage 1 for administration. *In vivo* evidence suggests that BM-MSCs can be affected by aging and are strongly associated with the mammalian life span and health conditions (12, 13). Recently, our group reported the negative effects of type 2 diabetes mellitus duration on the quality and metabolic function of autologous BM-MSCs (14). Hence, the variation in efficacy observed in a recent study using BM-MSCs for the treatment of frailty could be due to (1) the heterogeneous sources of BM-MSCs, which were derived from a wide age range of BM-MSC donors, (2) the *in vitro* culture of BM-MSCs in FBS (unknown

component, batch-to-batch variation, and animal-derived products), and (3) the aging-related effects of BM-MSCs.

An alternative source of allo-MSCs is needed to fill the gap in knowledge and provides another option regarding the strengths and limitations of applying BM-MSCs as a source of stem cells for the treatment of frailty using regenerative medicine. Therefore, in this study, we propose a randomized controlled phase I/II trial involving the allogeneic administration of umbilical cord-derived MSCs (UC-MSCs) for frailty.

### **Proposed molecular analysis of stem cell-associated efficacy in frailty treatment**

To evaluate the interrelationship between the efficacy of the treatment and the nature and function of UC-MSCs, several molecular experiments have been planned and will be performed at baseline and at 1 month, 3 months, 6 months and 9 months post-administration. (1) Analyses of cytokines, chemokines and growth factors in patient plasma using cytokine/chemokine/growth factor 45-plex human ProcartaPlex panel-1 will provide information on the inflammation status and immune response of the patient to UC-MSC administration. (2) The immunoregulatory properties of UC-MSCs on CD3<sup>+</sup> T lymphocytes of patients will be evaluated. (3) Measurements of cellular senescence by qPCR will be conducted with the CD3<sup>+</sup> cell population and senescence-associated b-galactosidase activity. (4) The metabolic profiles of CD3<sup>+</sup> cells will be evaluated using the Seahorse XF Cell Mito Stress Test Kit and Seahorse XF Cell Glycolysis Stress Test Kit (Agilent Technologies). (5) The expression of CD142 in different MSC products is associated with their procoagulant nature, which triggers instant blood-mediated inflammatory reactions (IBMIRs) via complex cascades of immunological reactions (15). A summary of the study procedure timeline and events is illustrated in Table 1 and Figure 2. The details of the study protocol are provided in Supplementary 1.

## Why is it important to picture aging hallmarks in such a complexed study design?

The accumulation of senescent cells is one of the hallmarks of human aging and is tightly related to telomere shortening and DNA damage. The former has a profound impact on cell proliferation and senescence because the shortening of telomeres has been suggested as a useful biomarker for cellular senescence and aging (16). Although the interrelationship between telomere shortening and frailty has been proposed as a potential biomarker of frailty at the clinical level, no correlation between telomere length and frailty condition has been reported (17, 18). In response to persistent DNA damage, cellular senescence occurs through activation of the INK4a/ARF (CDKN2a) locus, which leads to increased expression of  $p16^{INK4a}$ , a cell cycle kinase inhibitor. Studies have revealed that  $p16^{INK4a}$  is correlated with chronological age in both mice and humans, and the peripheral blood T-lymphocyte expression of  $p16^{INK4a}$  has been established as an indicator of human aging (19). Moreover, recent study suggested that expression of  $p16^{INK4a}$  is a biomarker of dysfunctional chondrocytes but is not essential for senescence-associated secretory phenotype (20). A meta-analysis of more than 300 genome-wide association studies demonstrated that the INK4a/ARF locus is generally linked to the highest number of age-associated pathologies, such as cardiovascular diseases, diabetes, glaucoma and Alzheimer's disease (21). In senescent cells, the structural change involved in the increased lysosomal content with the most widely used marker is the increased level of senescence-associated  $\beta$ -galactosidase activity (22). In this study, the expression of  $p16^{INK4a}$  and SA- $\beta$ -gal will be examined in patient CD3+ cells before and after UC-MSC administration.

## **Summary**

In this study, we emphasize the aims, significance, study design, and mechanistic investigation of the administration of allogeneic UC-MSCs for patients with frailty: a phase I/II clinical trial will be conducted to address the safety and potential efficacy of UC-MSC administration based on a set of clinical evaluations of frailty (including physiological and functional analyses and assessment of aging phenotypes). This study will also address the potential risk of thrombotic events after administration of the treatment in older subjects. The data accumulated during this study will provide invaluable insight into the mechanism of action of MSCs in improving the aging conditions in patients with frailty and will provide an alternative treatment to improve the impact of frailty in older patients to a certain extent.

## **Conflict of Interest**

The authors have no conflicts of interest to declare.

## **Funding**

This work was supported by the Vingroup Scientific and Clinical Research Grant (grant number: ISC.21.11).

## **Acknowledgments**

The authors would like to thank all patients involved in the study for their trust, understanding and willingness. We thank our collaborating clinicians at the Vinmec Health Care System for participating in this study.

## References:

1. Hoogendijk EO, Afilalo J, Ensrud KE, Kowal P, Onder G, Fried LP. Frailty: implications for clinical practice and public health. *Lancet*. 2019;**394**:1365-1375. doi: 10.1016/S0140-6736(19)31786-6
2. Dung V, Thi Mai Lan N, Thu Trang V, Xuan Cu T, Minh Thien L, Sy Thu N, *et al*. Quality of life of older adults in nursing homes in Vietnam. *Health Psychol Open*. 2020;**7**:2055102920954710. doi: 10.1177/2055102920954710
3. Collard RM, Boter H, Schoevers RA, Oude Voshaar RC. Prevalence of frailty in community-dwelling older persons: a systematic review. *J Am Geriatr Soc*. 2012;**60**:1487-1492. doi: 10.1111/j.1532-5415.2012.04054.x
4. Nguyen TV, Le D, Tran KD, Bui KX, Nguyen TN. Frailty in Older Patients with Acute Coronary Syndrome in Vietnam. *Clin Interv Aging*. 2019;**14**:2213-2222. doi: 10.2147/CIA.S234597
5. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, *et al*. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci*. 2001;**56**:M146-156. doi: 10.1093/gerona/56.3.m146
6. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell*. 2013;**153**:1194-1217. doi: 10.1016/j.cell.2013.05.039
7. Soysal P, Arik F, Smith L, Jackson SE, Isik AT. Inflammation, Frailty and Cardiovascular Disease. *Adv Exp Med Biol*. 2020;**1216**:55-64. doi: 10.1007/978-3-030-33330-0\_7
8. Lim JE, Son Y. Endogenous Stem Cells in Homeostasis and Aging. *Tissue Eng Regen Med*. 2017;**14**:679-698. doi: 10.1007/s13770-017-0097-3



9. Sun XL, Hao QK, Tang RJ, Xiao C, Ge ML, Dong BR. Frailty and Rejuvenation with Stem Cells: Therapeutic Opportunities and Clinical Challenges. *Rejuvenation Res.* 2019;**22**:484-497. doi: 10.1089/rej.2017.2048
10. Golpanian S, DiFede DL, Pujol MV, Lowery MH, Levis-Dusseau S, Goldstein BJ, *et al.* Rationale and design of the allogeneic human mesenchymal stem cells (hMSC) in patients with aging frailty via intravenous delivery (CRATUS) study: A phase I/II, randomized, blinded and placebo controlled trial to evaluate the safety and potential efficacy of allogeneic human mesenchymal stem cell infusion in patients with aging frailty. *Oncotarget.* 2016;**7**:11899-11912. doi: 10.18632/oncotarget.7727
11. Tompkins BA, DiFede DL, Khan A, Landin AM, Schulman IH, Pujol MV, *et al.* Allogeneic Mesenchymal Stem Cells Ameliorate Aging Frailty: A Phase II Randomized, Double-Blind, Placebo-Controlled Clinical Trial. *J Gerontol A Biol Sci Med Sci.* 2017;**72**:1513-1522. doi: 10.1093/gerona/glx137
12. Beane OS, Fonseca VC, Cooper LL, Koren G, Darling EM. Impact of aging on the regenerative properties of bone marrow-, muscle-, and adipose-derived mesenchymal stem/stromal cells. *PLoS One.* 2014;**9**:e115963. doi: 10.1371/journal.pone.0115963
13. Zhou S, Greenberger JS, Epperly MW, Goff JP, Adler C, Leboff MS, *et al.* Age-related intrinsic changes in human bone-marrow-derived mesenchymal stem cells and their differentiation to osteoblasts. *Aging Cell.* 2008;**7**:335-343. doi: 10.1111/j.1474-9726.2008.00377.x
14. Nguyen LT, Hoang DM, Nguyen KT, Bui DM, Nguyen HT, Le HTA, *et al.* Type 2 diabetes mellitus duration and obesity alter the efficacy of autologously transplanted bone marrow-derived mesenchymal stem/stromal cells. *Stem Cells Transl Med.* 2021. doi: 10.1002/sctm.20-0506

15. Moberg L, Johansson H, Lukinius A, Berne C, Foss A, Kallen R, *et al.* Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. *Lancet*. 2002;**360**:2039-2045. doi: 10.1016/s0140-6736(02)12020-4
16. Ben-Porath I, Weinberg RA. When cells get stressed: an integrative view of cellular senescence. *J Clin Invest*. 2004;**113**:8-13. doi: 10.1172/JCI20663
17. Mather KA, Jorm AF, Parslow RA, Christensen H. Is telomere length a biomarker of aging? A review. *J Gerontol A Biol Sci Med Sci*. 2011;**66**:202-213. doi: 10.1093/gerona/glq180
18. Haapanen MJ, Perala MM, Salonen MK, Guzzardi MA, Iozzo P, Kajantie E, *et al.* Telomere Length and Frailty: The Helsinki Birth Cohort Study. *J Am Med Dir Assoc*. 2018;**19**:658-662. doi: 10.1016/j.jamda.2018.05.011
19. Liu Y, Sanoff HK, Cho H, Burd CE, Torrice C, Ibrahim JG, *et al.* Expression of p16(INK4a) in peripheral blood T-cells is a biomarker of human aging. *Aging Cell*. 2009;**8**:439-448. doi: 10.1111/j.1474-9726.2009.00489.x
20. Diekman BO, Sessions GA, Collins JA, Knecht AK, Strum SL, Mitin NK, *et al.* Expression of p16(INK) (4a) is a biomarker of chondrocyte aging but does not cause osteoarthritis. *Aging Cell*. 2018;**17**:e12771. doi: 10.1111/acel.12771
21. Jeck WR, Siebold AP, Sharpless NE. Review: a meta-analysis of GWAS and age-associated diseases. *Aging Cell*. 2012;**11**:727-731. doi: 10.1111/j.1474-9726.2012.00871.x
22. Lee BY, Han JA, Im JS, Morrone A, Johung K, Goodwin EC, *et al.* Senescence-associated beta-galactosidase is lysosomal beta-galactosidase. *Aging Cell*. 2006;**5**:187-195. doi: 10.1111/j.1474-9726.2006.00199.x

## Figure legends

**Figure 1: Interrelationship among hallmarks of aging, clinical symptoms of frailty and potential regenerative therapy.** Aging people are constantly affected by oxidative stress and inflammatory responses throughout their later years. In an extreme condition of this interaction, frailty is developed and defined by five major criteria, including unintentional weight loss, exhaustion, slow gait speed, weak grip strength, and low physical activity. Frailty is strongly associated with the aging process, which includes six important hallmarks: genomic instability, deregulated proteostasis, nutrient sensing, metabolic malfunctions, cellular senescence, stem cell exhaustion, and compromised cellular communication. Emerging stem cell therapy provides a potential approach to tackle these problems by providing *ex vivo* cultured stem cells that are able to improve the frailty condition by multiple mechanisms.

**Figure 2: Proposed functional analysis to reveal the mechanism of action of UC-MSCs in the treatment of frailty.** (A) The UC-MSC line was derived from Vinmec Biobank and was intensively characterized and cultured under xeno-free and serum-free conditions. These cells will be expanded to reach the transplantation dose for 22 patients (2 administrations with  $1.5 \times 10^6$  cells/kg patient body weight) and cryopreserved for long-term storage. Cell viability, MSC marker, karyotype, product sterility (bacterial, fungal, and mycoplasma detection) and endotoxin will be assessed according to the Vietnamese Ministry of Health guideline and the International Society for Cell and gene Therapy (ISCT) criteria of MSCs. (B) Peripheral blood at baseline and at 1, 3, 6, and 9 months will be collected, and (C) detection of cytokines/chemokines/growth factors using procartaplex technology derived from patient plasma will be performed, whereas CD3+ cells will be isolated for: (D) immunological analysis, (E) cellular senescence, and (F) metabolic analysis using Seahorse XFe96 analyzer.

**Table 1: Study timeline and clinical procedures during the trial.** \* If the results of the screening phase for UC-MSc groups are within 30 days of UC-MSc administration, they will be automatically considered as the baseline level.

Study Procedure	Prescreening	Screening phase*	Baseline	1 month	3 months	6 months	9 months
UC-MSc administration <sup>1</sup>			<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>		
Medication treatment <sup>2</sup>			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Informed consent		<input checked="" type="checkbox"/>					
Inclusion and exclusion criteria		<input checked="" type="checkbox"/>					
Demographic information		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>			<input checked="" type="checkbox"/>
Patient's medical reports		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Vital signs <sup>3</sup> /physical examination		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Frailty evaluation <sup>4</sup>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Thrombotic analysis <sup>5</sup>			<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>		
Hematology analysis <sup>6</sup>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Infectious disease examination/test <sup>7</sup>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>				
Chest CT scan		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Chest X-ray		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Pulmonary function analysis <sup>8</sup>		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Adverse event evaluation			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

Monitoring of mortality/complications	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Blood sample for molecular and cellular analyses of frailty <sup>9</sup>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

<sup>1</sup>: Applies only for the UC-MSc group at baseline and 3 months.

<sup>2</sup>: The treatment medication administered to all testing groups included Hightamine (Hankook Korus Pharm, Korea), total calcium (Nugale Pharmaceutical, Canada), Bioflex (Ausbiomed, Australia) and Nootropil (UCB Pharma S.A., Germany).

<sup>3</sup>: The vital signs include body temperature, blood pressure, heart rate, respiratory rate, oxygen saturation, and patient body weight.

<sup>4</sup>: The frailty assessment and evaluation included analyses of physical activity (using the Community Healthy Activities Model Program for Seniors (CHAMPS) questionnaire), mobility (6-min walk test (6MWT)), handgrip strength (dynamometer measurement), exhaustion (multidimensional fatigue inventory questionnaire – MFI), level of pain in the knee (WOMAC) and quality of life (SF-36).

<sup>5</sup>: The thrombotic analysis included measurement of the D-dimer, fibrinogen, prothrombin (PT), thrombin (TT), and APTT levels prior to UC-MSc administration and at 1, 3 and 24 h postadministration.

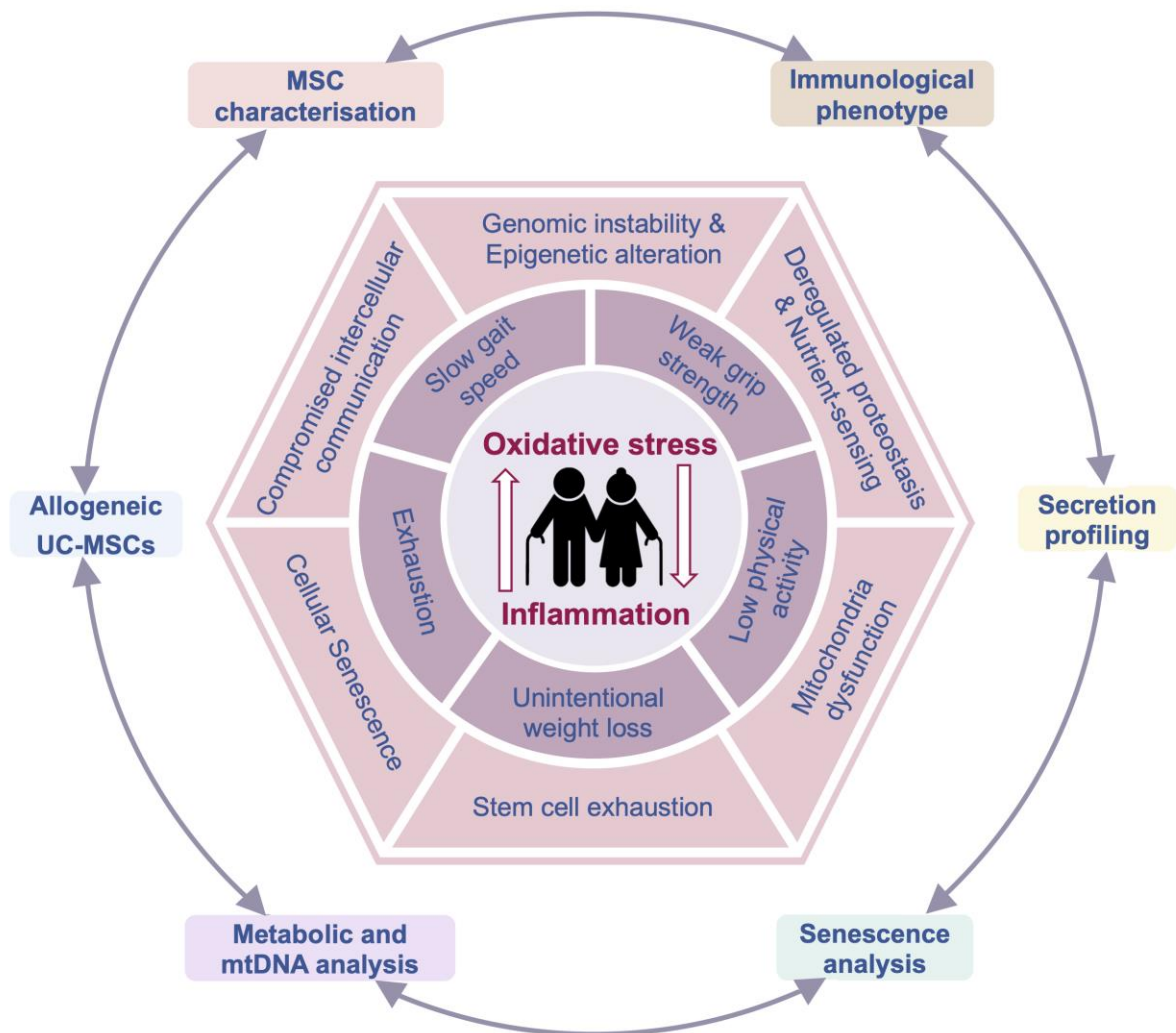
<sup>6</sup>: The hematological analysis included measurements of the white blood cell count, platelet count, red blood cell count, hemoglobin, and percentages of lymphocytes, neutrophils, monocytes, eosinophils, basophils, C-reactive protein, pro-BNP, and troponin-T.

<sup>7</sup>: Infectious diseases include hepatitis, syphilis, HIV, HBV, and tuberculosis.

<sup>8</sup>: The pulmonary function analysis includes assessments of the FEV<sub>1</sub>, FEV<sub>1</sub>/FVC, VC, TLC, and RV/TLC.

<sup>9</sup>: Blood samples will be collected for cellular and molecular analysis, including analyses of cytokines, chemokines and growth factors in the patient's plasma, CD3<sup>+</sup> cell isolation, evaluation of the immunoregulatory response of the patient's T lymphocytes, measurement of cellular senescence by qPCR, and metabolic analysis.

Accepted Manuscript



**Figure 1:** Duc M. Hoang et al.

Figure 2

