UNIVERSITE DE POITIERS

Faculté de médecine et de pharmacie

ANNEE 2022

THESE

POUR LE DIPLÔME D'ETAT

DE DOCTEUR EN MEDECINE

(décret du 16 janvier 2004)

Présentée et soutenue publiquement le 04 Mars 2022 à Paris

Par Monsieur Otriv Frédéric NGUEKAP TCHOUMBA

Traitement par cellules stromales mésenchymateuses dans le syndrome de détresse respiratoire aiguë lié au SARS-CoV-2

Composition du Jury :

Président:	Monsieur le Professeur	Jérôme LARGHERO
Membres:	Monsieur le Professeur émérite	Philippe MENASCHE
	Madame la Maîtresse de conférences	Michelle ROSENZWAJG
	Monsieur le Professeur	Arnaud THILLE
Directeur de thèse:	Monsieur le Professeur	Antoine MONSEL

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Le Doyen,

Année universitaire 2021 - 2022

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A la mémoire de mon cher oncle Ebenezer Nzonlia, décédé le 15 Avril 2020 au Cameroun

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Repose en paix.

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Abréviations

AMM : autorisation de mise sur le marché ARN : acide ribonucléique CD : cluster de différenciation CMH : complexe majeur d'histocompatibilité CO : cordon ombilical COVID-19 : coronavirus disease-2019 CS : cellule souche CSE : cellule souche embryonnaire CSM : cellule stromale mésenchymateuse CSM-CO : cellule stromale mésenchymateuse dérivée du cordon ombilical CSM-MO : cellule stromale mésenchymateuse dérivée de la moelle osseuse ECA2 : Enzyme de conversion de l'angiotensine 2 FiO₂ : fraction inspirée de dioxygène GM-CSF : Granulocyte-macrophage colony-stimulating factor HLA : human leucocyt antigen iCSP : cellule souche pluripotente induite IFN-I : interféron-I IFN-III : interféron-III IFN-γ : interféron-γ IL : interleukine IL-6 : interleukine-6 KGF: keratinocyte growth factor NaCl : chlorure de sodium NK : natural killer PaO₂: pression partielle de dioxygène

PaO₂/FiO₂ : pression partielle de dioxygène sur fraction inspirée de dioxygène

pDC : cellule dendritique plasmacytoïde

PNN : polynucléaire neutrophile

SARS–CoV-2: severe acute respiratory distress syndrom coronavirus-2

SDRA : syndrome de détresse respiratoire aigue

SOFA : Sequential Organ Failure Assessment

Teff : lymphocyte T effecteur TMPRSS2 : type 2 transmembrane serine protease TNF-α : tumor necrosis factor alpha Treg : lymphocyte T régulateur

I. Introduction

1. Atteinte respiratoire sévère du COVID-19 et dérégulation immunitaire

Le SARS-CoV-2 (*severe acute respiratory syndrome coronavirus-2*) est le virus responsable de la maladie coronavirus-2019 (COVID-19). La mortalité des formes les plus graves nécessitant une admission en réanimation est en moyenne de 30 à 40 % (1). La cause principale de décès dans le COVID-19 est une dérégulation du système immunitaire générant une réponse inflammatoire exagérée et mal contrôlée. Au niveau pulmonaire, elle entraîne entre autres, des lésions de la membrane alvéolo-capillaire pulmonaire caractéristique du syndrome de détresse respiratoire aiguë (SDRA), qui nécessite un support respiratoire par ventilation mécanique pouvant concerner jusqu'à 90 % des patients de réanimation (2). Le SDRA est une entité médicale définie par un œdème pulmonaire non cardiogénique dû à une agression alvéolaire par voie aérienne (atteinte directe) ou par voie systémique (atteinte indirecte). En pratique clinique, le diagnostic de SDRA repose sur quatre critères, qui sont énumérés dans le tableau 1 (3). Plusieurs mécanismes biologiques conduisant à la pneumonie et au SDRA au cours du COVID-19 sont maintenant bien décrits.

Syndrome de détresse respiratoire aiguë		
Installation aiguë	Dans la semaine suivant un accident clinique connu ou la survenue ou l'aggravation de symptômes respiratoires	
Imagerie thoracique	Opacités bilatérales ne pouvant entièrement s'expliquer par la présence d'une surcharge volémique, d'une atélectasie lobaire ou pulmonaire, ou de nodules	
Origine de l'œdème	Insuffisance respiratoire ne pouvant entièrement s'expliquer par une insuffisance cardiaque ou une surcharge hydrique. En l'absence de facteurs de risque, nécessité d'une évaluation objective (par exemple une échocardiographie) pour exclure une origine hydrostatique des infiltrats.	
Hypoxémie ^b		
Légère	200 mm Hg < PaO2/FiO2 ≤ 300 mm Hg (avec une PEP ou une PPC ≥5 cm H2O ^c)	
Modérée	100 mm Hg < PaO2/FiO2 ≤ 200 mm Hg (avec une PEP ≥5 cm H2O)	
Sévère	PaO2/FiO2 ≤ 100 mm Hg (avec une PEP ≥5 cm H2O)	

Tableau 1- Définition du syndrome de détresse respiratoire aiguë. Adapté de Force,	définition Al	RDS Task, et
al. JAMA 2012		

Abréviations : FiO2, fraction inspirée en oxygène ; PaO2, pression partielle de l'oxygène ; PEP, pression expiratoire positive ; PPC, pression positive continue.

^a Radiographie ou tomodensitométrie thoracique

^b Si l'altitude est supérieure à 1000 m, un facteur de correction doit être utilisé et calculé comme suit : PaO2/FiO2 x pression atmosphérique/760

^c La PEP/PPC peut être délivrée de façon non invasive uniquement dans le groupe de patients ayant une hypoxémie légère

A. Tropisme pulmonaire du SARS-CoV-2

Le SARS-CoV-2 est un virus à ARN de la famille des coronavirus avec transmission interhumaine par aérosol. Il pénètre dans le poumon par inhalation via les voies aériennes et cible les cellules épithéliales, endothéliales, macrophagiques (4–7). La sérine protéase transmembranaire de type 2 (acronyme anglais TMPRSS2) des cellules humaines clive et expose le site de fusion de la protéine spike du virus (8). La protéine spike peut alors se lier à l'enzyme de conversion de l'angiotensine 2 (ECA2) exprimée à la surface des cellules hôtes, ceci permet la fusion des membranes et la pénétration du virus dans ces cellules (4,8). L'ECA2 et le TMPRSS2 sont exprimés sur les cellules épithéliales pulmonaires notamment les pneumocytes de type 2, ce qui favorise la tropisme pulmonaire du virus (9–11). Outre sa capacité à utiliser la protéine TMPRSS2 pour catalyser l'interaction entre la protéine spike et l'ECA2, le SARS-CoV-2 à la différence du SARS-CoV peut se servir d'autres protéases humaines comme la Furine (12–14). Enfin, comparé à d'autres tissus, l'épithélium respiratoire exprime à sa surface un glycocalyx (couche de polysaccharides liés de façon covalente à la membrane cellulaire) qui facilite le piégeage du virus sur la muqueuse mais aussi l'interaction entre la protéine spike et l'ECA2 (15). Ces multiples mécanismes d'invasion cellulaire pourraient participer à la plus grande infectiosité de ce virus et à la sévérité de l'atteinte respiratoire. Les pièces autopsiques pulmonaires de patients décédés de COVID-19 montrent un dommage alvéolaire diffus (hémorragie intra-alvéolaire, membranes hyalines, infiltrat neutrophilique massif interstitiel et alvéolaire, épaississement des septa interlobulaires) avec œdème pulmonaire, dépôt de collagène et fibrine, mais aussi une destruction endothéliale associée à de la thrombose (5,16,17). L'invasion virale des cellules hôtes s'accompagne d'une mort cellulaire et d'une infiltration par les cellules immunitaires inflammatoires (5,18).

B. Infiltration pulmonaire par les cellules immunitaires inflammatoires

Il s'agit principalement de cellules myéloïdes, en particulier les polynucléaires neutrophiles (PNNs) et les monocytes/macrophages (5,19,20). Le PNN joue un rôle délétère dans le SDRA lié au COVID-19, en libérant des molécules telles que les dérivés réactifs de l'oxygène (radicaux superoxyde et peroxyde) ou des enzymes lytiques contenues dans ses granules qui participent au dommage alvéolaire (21,22). Il amplifie également la réponse inflammatoire par la libération de cytokines et chimiokines (23,24). Par ailleurs, le SARS–CoV-2 induit directement le phénomène de nétose, ce qui permet le relargage de facteurs solubles tels que la

myélopéroxydase et l'élastase participant à la destruction de la barrière alvéolo-capillaire (19,25,26). Dans des pathologies inflammatoires comme le lupus ou dans l'athérosclérose, la nétose induit la sécrétion de cytokines pro-inflammatoires (par exemple l'IL-1β) par les macrophages (27,28). La nétose semble être impliquée dans la thrombose pulmonaire associée au COVID-19 (26), ceci via l'activation de l'hémostase primaire et secondaire (29–32). Le monocyte/macrophage participe également à la destruction des cellules infectées via ses enzymes lytiques et la phagocytose. Il relargue également des chimiokines et cytokines pro-inflammatoires (33). Au total, l'infiltration cellulaire par les monocytes et les PNNs joue un rôle direct dans la destruction de la membrane alvéolo-capillaire, l'amplification du relargage cytokinique et la thrombose associée au COVID-19.

C. Activation de la cascade du système du complément

Comparés à la forme modérée de COVID-19 ou à des sujets sains, les patients ayant été atteints d'une forme sévère de la maladie ont une élévation significative de la fraction sérique C5a (anaphylatoxine libérée par la cascade d'activation du complément) (34,35). Les biopsies pulmonaires de patients décédés par infection au SARS-CoV-2 présentent des dépôts épithéliaux et endothéliaux de fractions activées du complément notamment du complexe d'attaque membranaire (fraction activée de complément responsable de la mort cellulaire) (5,36). Ces dépôts sont significativement plus élevés que chez des personnes décédées de: pathologie non pulmonaire, SDRA lié à la grippe ou SDRA d'origine bactérienne (5).

Le SARS-CoV-2 active directement le système du complément (voie des lectines) (34,37). De plus, la cascade du complément peut être initiée directement par la nétose ou par la protéine *spike* du virus (voie alterne) (31,38) et par les complexes immuns ou les corps apoptotiques (voie classique) (39,40). Le système du complément participe donc au SDRA par l'induction de la mort des cellules épithéliales et endothéliales via le complexe d'attaque membranaire (5), l'activation et le recrutement des PNNs et des monocytes via ses anaphylatoxines (35). Enfin, le complément via l'activation de la nétose participe aussi à la thrombose (31).

D. Dérégulation de la balance entre les lymphocytes effecteurs et régulateurs

Chez les patients ayant un COVID-19 nécessitant une hospitalisation, il existe un excès de lymphocyte T CD4+ effecteur (Teff) et un défaut de lymphocyte T CD4+ régulateur (Treg) (41). De plus, les travaux de Qin et al (42) montrent que les patients ayant des formes sévères ont

une réduction significative du nombre de Tregs dans le sang périphérique par rapport aux sujets sains et aux COVID-19 non sévères.

Les Teffs sont connus pour avoir un rôle dans la libération de cytokines pro-inflammatoires comme le GM-CSF (facteur de croissance des monocytes) (43) et la destruction des cellules infectées. Les Tregs jouent aussi un rôle trophique en favorisant la réparation alvéolaire dans le SDRA via la synthèse de médiateurs tels que l'amphiréguline et le KGF (keratinocyte growth factor) (44–46). Les Tregs participent également à la clairance des cellules inflammatoires (47,48). Il a été montré chez les patients ayant un SDRA non lié au COVID-19, qu'une élévation du ratio entre Teff et Treg s'accompagne d'une augmentation significative de la mortalité à moyen (28 jours) et à long terme (90 jours) (49,50). Une dérégulation de la balance entre Teff et Treg participe donc à l'inflammation pulmonaire et au SDRA lié au COVID-19.

E. Déficit immunitaire dans les formes sévères de COVID-19

Comparé à d'autres virus respiratoires tels que le virus *Parainfluenza* ou le virus respiratoire syncytial, le SARS-CoV-2 entraine un faible niveau d'expression de transcrits de l'IFN-I et IFN-III et des gènes stimulés par l'IFN-I et l'IFN-III (51–54). Il y a une baisse significative de l'activité interféron et du taux sanguin d'IFN-I chez les patients sévères comparativement à la forme modérée (53,55). En revanche, on observe une forte expression de cytokines pro-inflammatoires telles que l'IL-1 β , IL-6, IL-12 ou le TNF- α chez ces patients sévères (52). Le déficit de la réponse IFN-I et III préexiste à la détérioration clinique et l'admission en réanimation (53,54). Il s'associe à un allongement de la clairance virale et du séjour en réanimation (53,54).

Environ 10% des formes sévères de COVID-19 ont des auto-anticorps de type IgG anti-IFN-I, 3.5% ont une anomalie génétique de la voie de synthèse ou de signalisation de l'IFN-I. Ces anomalies sont absentes chez les patients ayant des formes modérées (56,57,55,58). De plus, le SARS-CoV-2 a la propriété intrinsèque (en comparaison au virus *Influenza*) d'inhiber la biosynthèse protéique d'IFN-I notamment dans les cellules dendritiques plasmacytoïdes (pDCs), cellules clés pour la production d'IFN-I dans les viroses aigües (57,59).

Les patients avec un COVID-19 sévère ont une réduction du nombre de pDCs dans le poumon et une augmentation du ratio entre les cellules dendritiques conventionnelles et les pDCs dans le sang (20,60). A défaut de produire une réponse IFN-I efficace, ces pDCs dysfonctionnelles relarguent un excès de de cytokines pro-inflammatoires (par exemple l'IL-6 et le TNF- α) (61).

La dérégulation des cellules dendritiques (pivot de l'immunité adaptative T antivirale) s'associe donc à: une lymphopénie T plus marquée dans les formes sévères, un défaut d'activation, de prolifération et une exhaustion des lymphocytes T (51,53,60,62). En résumé, le déficit IFN-I et III dépend d'au moins 3 mécanismes : l'auto-immunité, le déficit génétique et la dérégulation des pDCs induite par le SARS-CoV-2. Plus récemment, il a été identifié chez des patients ayant des formes sévères de COVID-19, de nouveaux auto-anticorps pathogènes dirigées contre une variété de protéines clés de la réponse antivirale, par exemple la sous-unité béta du récepteur à l'IL-18 (induction d'une réponse cytotoxique T et NK robuste) (63).

Ainsi le SARS-CoV-2 déclenche une activation de l'immunité innée avec libération de médiateurs pro-inflammatoires telles que l' IL-1 β , IL-6, IL-12, IL-18 ou le TNF- α par l'épithélium et les macrophages pulmonaires (5,33,64). Cette étape aboutit à l'infiltration par les cellules immunitaires inflammatoires et à l'activation du complément dans l'optique d'une clairance virale en concertation avec l'immunité adaptative. Un déficit de la réponse antivirale favorise la persistance du virus, la destruction accélérée de la membrane alvéolo-capillaire par amplification de l'infiltrat de cellules inflammatoires délétères et la potentialisation de l'orage cytokinique. Des niveaux élevés de médiateurs inflammatoires tels que l'IL-6 ou le TNF-α sont des biomarqueurs de pneumonie COVID-19 sévère avec pronostic défavorable (65,66). La gravité du SDRA lié au COVID-19, malgré un orage cytokinique plus modéré que dans d'autres états inflammatoires (67–70), peut s'expliquer par la persistance du virus cytopathique du fait d'un déficit immunitaire. La dérégulation de la réponse inflammatoire permet donc la destruction de la membrane alvéolo-capillaire conduisant au SDRA (voir figure 1) (2,71). L'administration systémique de corticostéroïdes a montré une réduction du taux de mortalité à 28 jours des patients avec SDRA associé au COVID-19 (72). De même, il a été rapporté que les anti-IL-6 améliorent le pronostic (survie et jours sans soin de support d'organes) (73). La rareté de thérapies efficaces dans les formes graves de COVID-19, expliquent probablement la mortalité toujours élevée du SDRA lié au SARS–CoV-2 (74) et justifie la recherche continue de traitements à visée immunomodulatrice. Parmi ceux-ci, les CSMs suscitent un intérêt croissant.



2. Cellules stromales mésenchymateuses : potentiel thérapeutique dans le SDRA

A. Définition et sous-types des cellules souches

Les cellules souches (CS) sont des cellules non spécialisées qui ont la capacité d'autorenouvellement et un potentiel de différenciation en des cellules spécifiques d'un tissu ou d'un organe (75). Elles existent chez l'embryon, le fœtus et l'adulte. Sur la base de leur pouvoir de différenciation décroissant, il existe quatre types de CS: totipotente, pluripotente, multipotente et unipotente (75). En fonction de leur origine pour la recherche et l'utilisation clinique, les CS peuvent être classées en CS : embryonnaires (CSE), issues du cordon ombilical (CO) et adultes (voir figure 2).

Le zygote abouti au 4^e jour post-fécondation à un amas cellulaire appelé *morula*. Toutes les cellules de la morula sont totipotentes, c'est-à-dire capables de se différencier à la fois en tous les types cellulaires d'un individu (corps entier) et aussi en ses annexes embryonnaires (par exemple le placenta). La morula se creuse d'une cavité liquidienne entre le 5^e et 6^e jour et devient le blastocyste. Il est composé d'une couche cellulaire bordante, d'une cavité interne liquidienne (blastocèle) et d'une masse cellulaire interne (voir figure 2). Les cellules bordantes se différencient et constitueront le placenta, essentiel pour l'implantation utérin de l'embryon. C'est dans la masse cellulaire interne du blastocyste qu'on trouve les CSE pluripotentes à l'origine de toutes les cellules d'un individu (à l'exception de ses annexes embryonnaires comme le placenta). Après l'implantation utérine, la masse cellulaire interne génère des CS multipotentes qui ne peuvent être que la source de cellules d'un tissu ou d'un organe spécifique. Après la naissance, il persiste un pool de CS multipotentes et unipotentes chez l'enfant et l'adulte. Une CS unipotente ne peut donner qu'un seul type de cellule différenciée (mature). Les CS sont utiles pour l'organogenèse et sont aussi la source de nouvelles cellules pour la croissance et la régénération des tissus. Outre le processus naturel de différenciation du zygote qui génère les CS, des CS pluripotentes peuvent être produites expérimentalement à partir d'une cellule adulte mature : on parle alors de CS pluripotente induite (iCSP) (75).



Figure 2- Origine des cellules souches pour la recherche et l'utilisation en clinique

B. Cellules stromales mésenchymateuses issues du cordon ombilical

Le CO permet les échanges biologiques entre la mère et le fœtus. Il contient 2 artères et 1 veine dans une matrice gélatineuse de tissu conjonctif (gelée de Wharton), le tout étant enveloppé par l'épithélium de revêtement de la membrane amniotique. La découverte de biomarqueurs des cellules souches a permis d'admettre qu'outre son rôle dans la protection mécanique des vaisseaux du CO, la gelée de Wharton est une source riche de cellules souches multipotentes, notamment les cellules stromales mésenchymateuses (CSMs) (76,77). Les biomarqueurs minimaux communs à tous les CSMs sont : la positivité pour le CD73, CD105, CD90 et négativité pour le CD34 (marqueur de progéniteurs hématopoïétiques primitifs et de cellules endothéliales), CD45 (marqueur pan-leucocytaire), CD79a ou CD19 (marqueurs de lymphocytes B), CD14, CD11b (marqueurs de macrophages monocytes), HLA de classe II (HLA DR). De plus, les CSMs doivent être adhérentes au plastique et pouvoir se différencier in vitro en ostéoblastes, adipocytes, chondroblastes (tableau 2) (78). Elles suscitent un intérêt du fait de leur potentielle application en médecine régénérative ou dans les maladies inflammatoires.

Tableau 2- Résumé des critères d'identification des cellules stromales mésenchymateuses. De Dominici, M. L.B. K., et al. Cytotherapy 2006.

tions
e (≤2%+)
r CD11b
or CD19
R
chondroblasts
re)

C. Action immunomodulatrice des cellules stromales mésenchymateuses

Une caractéristique majeure des CSMs est leur privilège immun. En effet, elles ont généralement une expression réduite de CMH-I et pas d'expression du CMH-II qui sont cruciaux pour la réponse des lymphocytes T alloréactifs. Elles expriment l'HLA-G qui est immunomodulateur sur les cellules immunitaires (79). De plus, ces CSMs expriment faiblement d'autres molécules classiquement nécessaires à la costimulation des Teffs, notamment le CD40, CD80/86 (79–81). Toutes ces propriétés participent à la faible immunogénicité des CSMs chez le receveur après leur transplantation.

Dans un contexte inflammatoire, les CSMs sont immunomodulatrices (voir figure 4) :

-*Sur les lymphocytes T effecteurs et NK*: Inhibition de l'activation, la prolifération et de la cytotoxicité des Teffs et des cellules NK (79,82,80).

-*Sur les cellules dendritiques*: Inhibition de la différenciation des cellules dendritiques à partir des monocytes (83). Les CSMs bloquent la maturation de cellules dendritiques et réduisent aussi la sécrétion de cytokines pro-inflammatoires (par exemple IL-12, IFN-γ) (84,85).

-*Sur les monocytes et les macrophages*: La sécrétion d'HGF (*hepatocyte growth factor*) et d'IL-6 par les CSMs instruit les monocytes à produire de l'IL-10, cytokine immunorégulatrice (86).

-*Sur les lymphocytes T régulateurs*: Stimulation et différenciation des lymphocytes T CD4+ en Tregs (82,83). Ces cellules jouent une rôle immunomodulateur et un rôle trophique pour la cicatrisation de lésions pulmonaires aiguës (87).

-*Sur les lymphocytes B* : L'effet des CSMs est dépendant du microenvironnement. La présence d'IL-2 semble conférer aux CSMs une capacité immunostimulatrice (88,89), tandis que l'IL-4 semble procurer aux CSMs un effet immunomodulateur sur les lymphocytes B (90,91).



Figure 4- Mécanismes d'immunomodulation par les cellules stromales mésenchymateuses issues du cordon ombilical dans un contexte inflammatoire. De Mebarki, Miryam, et al. Stem Cell Research & Therapy 2021. *IL-10 : interleukine-10 ; PGE2 : prostaglandine-2 ; TGF8 : transforming growth factor-8 ; IDO : Indoleamine 2,3dioxygenase ; HLA-G6 : Human leucocyt antigen-G6 ; TSG6 : TNF-stimulated gene-6 ; MCP-1 : Monocyte chemoattractant protein-1 ; HGF : hepatocyte growth factor ; IL-6 : interleukin-6.*

D. Cellules stromales mésenchymateuses et SDRA d'origine infectieuse

Dans les modèles animaux de SDRA infectieux, l'injection de CSMs s'accompagne d'une réduction significative de la charge bactérienne pulmonaire, de la bactériémie (92–94). De plus, il y a une inhibition de la croissance de bactéries cultivées en présence des CSMs (92,94). Ces cellules soutiennent également la réparation tissulaire en améliorant la: clairance de l'œdème alvéolaire, perméabilité intercellulaire, fibrose, régénération de pneumocytes, production de surfactant, survie (95–98). Les CSMs ont donc des propriétés antimicrobiennes (sécrétion de peptides antibactériens, et stimulation de la phagocytose) et réparatrices, en plus de leur effet immunomodulateur dans le SDRA.

Le traitement par CSM dans le SDRA non lié au SARS-CoV-2 s'est avéré bien toléré chez le patient de réanimation avec défaillances d'organes et réduit les biomarqueurs de lésion endothéliale (99–101). Il convient de noter que la thérapie par CSM est validée dans: les fistules cutanéo-digestives anales liées à la maladie de Crohn (AMM européenne), la maladie aiguë du greffon contre l'hôte en Europe, au Japon et au Canada (102,103), le traumatisme de moelle épinière (au japon) (104) et il fait l'objet de nombreux essais cliniques dans les maladies auto-immunes.

E. Avantages des cellules stromales mésenchymateuses du cordon ombilical

Ces CS peuvent être obtenues à partir de tissus périnataux (par exemple le CO ou le placenta), ou de tissus adultes (par exemple le tissu adipeux ou la moelle osseuse (MO)) (105). L'usage de CSM-CO présente de multiples avantages en comparaison à d'autres types de CS :

- Contrairement aux CSE, la collecte de CSM-CO ne soulève aucune préoccupation éthique liée à la manipulation ou la destruction d'embryons.

-Les CS adultes (par exemple les CSM-MO) et les iCSPs nécessitent un prélèvement de tissu chez un donneur sain. La procédure de prélèvement peut exposer ce dernier à des complications. Les CSM-CO sont collectées sur le cordon après l'accouchement, cette procédure a une innocuité parfaite pour la mère et le nouveau-né.

-Les iCSPs et CSE en raison de leur pluripotence sont sujets à la genèse de tumeurs type tératome ; ceci crée un risque de sécurité sanitaire en pratique clinique contrairement à l'utilisation de CSM-CO qui ne présentent pas ce risque (84,106,107).

-Les CS adultes sont entrelacées avec des cellules différenciées dans les tissus adultes. La collecte de CS à partir de tissus adultes possède un rendement inférieur à celui des CSM-CO. De plus, les CSM-CO ont un taux de prolifération plus élevé que les CSMs issues de la moelle (CSM-MO) (108). Ceci est pertinent en cas d'un besoin de traitement urgent tel qu'une pandémie.

-En comparaison aux CSM-MO, les CSM-CO expriment moins de molécules costimulatrices de lymphocytes T (CD80/86) et d'HLA-II même dans des conditions inflammatoires qui sont capables d'induire ces molécules (79). De plus, les CSM-CO expriment l'HLA-G6 (HLA immunomodulatrice) ce qui réduit le risque d'inflammation délétère lié à l'alloréactivité (79). Cette bonne immunotolérance autorise donc un traitement par CSM-CO allogéniques sans immunosuppresseur.

-Les CSM-CO ont des effets bénéfiques supérieurs aux CSM-MO dans les modèles précliniques d'infection virale pulmonaire (109).

Ainsi, ces nombreux avantages confèrent une place de choix à l'usage des CSM-CO en comparaison à d'autres sources potentielles de CSMs.

3. Objectif de mon travail de thèse

Compte tenu du défi mondial soulevé par l'impact sanitaire de la maladie COVID-19 et la quête permanente de traitements curatifs efficaces, les CSM-CO semblent être une thérapie potentielle à étudier dans le SDRA lié au SARS-CoV-2. Les caractéristiques attrayantes des CSM-CO précédemment discutées fournissent une logique thérapeutique pour évaluer ce traitement dans le SDRA induit par le SARS-CoV-2, car il pourrait avoir un spectre d'action plus large que la plupart des biothérapies, qui ont généralement un nombre limité de cibles moléculaires. En outre, leur migration à prédominance pulmonaire après perfusion intraveineuse (110–112) et leur sécurité clinique bien établie chez des patients de réanimation (113) plaident également en faveur de leur administration aux patients présentant cette pathologie. La majorité des essais cliniques publiés sont faits chez des patients étiquetés « sévères » mais sans présence des critères de SDRA ou de ventilation invasive (114–119). De plus, ces études évaluant le traitement par CSMs dans l'infection COVID-19 ne renseignent pas d'information sur la distribution entre les groupes de patients de facteurs importants comme la consommation de tabac, l'utilisation d'anticoagulants ou encore l'utilisation d'autres immunothérapies concomitantes (par exemple les anticorps monoclonaux anti-IL-6 ou de la corticothérapie) rendant difficile l'imputabilité des CSMs sur l'évolution clinique observée (114–120). L'étude monocentrique randomisée, contrôlée, double aveugle, contre placebo de Lanzoni et al (12 patients par groupe) a suggéré une réduction significative de la mortalité à 28 jours (critère de jugement secondaire) chez les patients traités par CSM-CO ayant un SDRA lié au COVID-19 selon les critères de Berlin (121). Par ailleurs, il existe dans cette étude un défaut d'information sur la répartition dans les groupes de patients, de facteurs comme : le type de support ventilatoire et ses paramètres, la temporalité par rapport au début des symptômes ou par rapport au diagnostic de SDRA, la présence de défaillance d'organes autres que le poumon. Ces facteurs sont des biais potentiels rendant complexe l'interprétation de cet essai clinique. L'essai STROMA-CoV-2 multicentrique, en double aveugle, randomisé et contrôlé par placebo (phase 2b) a été conçu pour répondre à ces limitations et déterminer si des perfusions intraveineuses répétées de CSMs, dérivés de la gelée de Wharton, au stade précoce du SDRA induit par le SARS-CoV-2 pourraient améliorer sa résolution et avoir un impact sur les biomarqueurs circulants.

 II. <u>Résultats du travail de thèse</u>: Manuscrit de l'article original soumis et accepté dans Critical care le 14 février 2022.

Ma contribution pour la réalisation de l'étude STROMA-CoV-2 a été la suivante (voir page 20 du manuscrit accepté-partie « Authors's contribution », individu intitulé « ONT ») :

- Inclusion des patients de réanimation dans l'étude (centre Pitié Salpêtrière)
- Organisation du rapatriement des prélèvements sériques depuis les divers centres vers la biobanque du service de biothérapies de la Pitié Salpêtrière
- Création et gestion de la sérothèque STROMA-CoV-2 (>500 échantillons sériques)
- Organisation logistique pour l'envoi des prélèvements aux laboratoires de virologie du CHU Pitié Salpêtrière et d'immunologie du CHU Bichat.
- Réalisation du dosage sérique de l'ensemble des biomarqueurs inflammatoires (ELISA QUANTIKINE et LUMINEX) pour tous les patients aux différents points de temps.
- Analyse multiomique et interprétation des dosages de biomarqueurs inflammatoires
- Analyse et interprétation des données cliniques
- Rédaction du manuscrit (matériel et méthodes, résultats , discussion)
- Relecture du manuscrit

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2	multicenter randomized double-blind trial.
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ABSTRACT

Background: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)-induced acute respiratory distress syndrome (ARDS) causes high mortality. Umbilical cord-derived mesenchymal stromal cells (UC-MSCs) have potentially relevant immune-modulatory properties, whose place in ARDS treatment is not established. This phase 2b trial was undertaken to assess the efficacy of UC-MSCs in patients with SARS–CoV-2-induced ARDS. Methods: This multicentre, double-blind, randomized, placebo-controlled trial (STROMA-CoV-2) recruited adults (≥18 years) with SARS–CoV-2-induced early (<96 hours) mild-to-severe ARDS in 10 French centres. Patients were randomly assigned to receive three intravenous infusions of 10⁶ UC-MSCs/kg or placebo (0.9% NaCl) over 5 days after recruitment. For the modified intention-to-treat population, the primary endpoint was the partial pressure of oxygen to fractional inspired oxygen (PaO₂/FiO₂)-ratio change between baseline (day (D) 0) and D7. **Results:** Among the 107 patients screened for eligibility from April 6, 2020, to October 29, 2020, 45 were enrolled, randomized and analyzed. PaO₂/FiO₂ changes between D0 and D7 did not differ significantly between the UC-MSCs and placebo groups (medians [IQR] 54.3 [-15.5 to 93.3] vs 25.3 [-33.3 to 104.6], respectively; ANCOVA estimated treatment effect 7.4,

18 95% CI –44·7 to 59·7; P=0.77). Six (28.6%) of the 21 UC-MSCs recipients and six of 24

19 (25%) placebo-group patients experienced serious adverse events, none of which were related20 to UC-MSCs treatment.

Conclusions: D0-to-D7 PaO₂/FiO₂ changes for intravenous UC-MSCs- *vs* placebo-treated
adults with SARS-CoV-2-induced ARDS did not differ significantly. Repeated UC-MSCs
infusions were not associated with any serious adverse events during treatment or thereafter
(until D28). Larger trials enrolling patients earlier during the course of their ARDS are needed
to further assess UC-MSCs efficacy in this context.

Trial registration: NCT04333368. Registered 01 April 2020,

2 https://clinicaltrials.gov/ct2/history/NCT04333368.

3 Key words: severe acute respiratory syndrome coronavirus-2; acute respiratory distress

syndrome; umbilical cord-derived mesenchymal stromal cells; good-manufacturing practice

BACKGROUND

30-40% and necessitating intensive care unit (ICU) admission [1]. A major root cause of those deaths is uncontrolled immune-system dysregulation, leading to, among others, alveolocapillary membrane damage evolving into acute respiratory distress syndrome (ARDS), which requires mechanical ventilation in up to 90% of ICU patients [2]. One of the hallmarks of COVID-19-associated ARDS is a dysregulated immune response, characterized by a shift of immune cells and their secreted cytokines towards an inflammatory pattern [3]. So far, systemic corticosteroid administration has been shown to lower the 28-day mortality rate of critically-ill COVID-19 patients [3]. Likewise, interleukin (IL)-6-receptor blockers have been reported to improve outcomes (organ-support-free days and survival) [4]. However, the challenges of implementing the vaccination strategy and the emergence of new viral variants still contribute to the persistently high mortality of patients with SARS-CoV-2induced ARDS [5]. Those findings justify the ongoing quest for new therapies, among which mesenchymal stromal cells (MSCs) are gaining increased interest.

MSCs have well-documented, anti-inflammatory and immune-modulatory properties [6], which have supported their use in treating diseases whose pathophysiologies harbor a major inflammatory component [7]. Specifically, their anti-apoptotic, anti-oxidative, and tissuereparative properties, diminishing lung vascular and epithelial permeability to proteins, and enhancing clearance of alveolar oedema fluid, have been demonstrated [8]. Furthermore, their capacity to temporarily evade the immune system [9] allows an allogeneic use, which streamlines the logistics of their clinical implementation. In addition, the results of a recent systematic review and meta-analysis of 55 clinical trials demonstrated their satisfactory safety profile [10]. Importantly, MSCs require priming by inflammatory signals to activate their

immunomodulatory functions [11], and some of those signals have been shown to be involved in the pathophysiology of ARDS. Furthermore, their predominant pulmonary lodging following intravenous infusion also argue in favour of administering them to patients with ARDS. As a matter of fact, MSCs have been administered intravenously in phase 1 and 2 clinical trials in more than 150 critically-ill ARDS patients with excellent results in terms of clinical tolerance and even some benefits on the modulation of inflammation biomarkers [12– 14]. The combination of those features makes MSCs appealing candidates for treating SARS-CoV-2-induced pulmonary inflammation, as they might have a broader spectrum of action than drugs, which usually have a more limited number of targets. While MSCs can be harvested from various tissue sources, those from the umbilical cord (UC) Wharton's jelly have distinct advantages over bone marrow- or fat tissue-derived MSCs: easy and non-invasive harvesting procedure, good clinical tolerance [15], excellent in vitro scalability and slower time to senescence. Perhaps of greater relevance in the specific context of COVID-19-induced pulmonary damage, UC-MSCs are credited with stronger angiogeneic [16,17] and immunomodulatory properties [18,19]. This is well-exemplified by the recent case report of a patient with COVID-19 respiratory failure, who received an intravenously infused of UC-MSCs, and whose in-depth immune profiling of peripheral blood and bronchoalveolar fluid lavage samples revealed: normalization of the circulating T-lymphocytes count, and reductions of inflammatory myeloid cells, serum levels of proinflammatory cytokines and lung-infiltrating inflammatory neutrophils, while circulating monocytes and low-density gradient neutrophils acquired immunosuppressive functions [20]. These UC-MSCs characteristics, possibly attributable to their more primitive origin compared with adult tissue-derived MSCs [18], might explain why those of cord origin were the most widely used according to a recent review of registered trials testing MSCs in COVID-19 patients [21]. In the specific context of SARS-CoV-2-induced severe ARDS, another reason for using UC-

MSCs, is that they do not express the angiotensin-converting enzyme-2 receptor [22], unlike
 MSCs originating from other tissue types [23].

Studies testing UC-MSCs in COVID-19 patients published so far have consisted of anecdotal case reports [24,25], small-sized non-randomized or open-label studies [22,26–29] and only two recent, single-centre, double-blind, placebo-controlled trials [30,31]. Overall, those studies' results confirmed the excellent tolerance of intravenous MSC infusions and suggested improved clinical outcomes, although their interpretation is complicated by the broad heterogeneity of patients' pre-treatment profiles, therapeutic doses and timing of treatment administration, cell tissue source, the different passage numbers at which cells were collected and the diversity of additional treatments, which generate strong background noise. We therefore designed this multicentre, double-blind, randomized, placebo-controlled STROMA-CoV-2 trial was designed to determine whether repeated intravenous infusions of UC-MSCs derived from Wharton's jelly during the early stage of SARS-CoV-2-related ARDS could improve its resolution and impact circulating levels of biomarkers.

METHODS

A detailed description of the methods and the full clinical trial protocol are provided in theAdditional file 1.

20 Study design

The multicentre, double-blind, randomized, placebo-controlled STROMA–CoV-2 trial was designed to compare the intravenous infusion of UC-MSCs *vs* saline placebo as add-on therapy for the management of SARS–CoV-2-induced ARDS. The study was conducted in ten ICUs in eight French university hospitals. The National Review Board of Île-de-France III approved the trial (CNRIPH 20.03.26.39722) that was authorized by the French National Agency for Medicines and Health Products Safety (EudraCT 2020-001287-28). The trial is registered with ClinicalTrals.gov identifier NCT04333368. A Data-Safety–Monitoring Board reviewed serious adverse events and the results after 10, 20 and 40 patients had been enrolled.

Patients

Eligible patients had Berlin criteria-defined ARDS (mild-to-severe) for <96 hours, reverse transcriptase–polymerase chain reaction (RT-PCR)-confirmed SARS–CoV-2 infection, and were receiving respiratory support (invasive or non-invasive mechanical ventilation, and/or high-flow nasal oxygenation, with positive end-expiratory pressure equivalent \geq 5 cmH₂O). Patients with pulmonary embolism, immunocompromised status, liver disease, chronic lung disease or cancer were excluded. The detailed list of exclusion criteria is given in Table 1. Written informed consent was obtained from patients or a legally designated representative.

14 Randomization and blinding

Subjects were initially randomized at a 1:2 ratio to receive either the total dose of 3×10^6 UC-MSCs/kg in 150 mL of 0.9% NaCl/0.5% albumin or placebo (150 mL of 0.9% NaCl) over 5 days, which was modified by a protocol amendment to 1:1 when patients became scarce at the end of the first wave. Randomization was stratified according to age (≤ 70 vs. >70 years) and the inclusion-day (D0) Sequential Organ-Failure Assessment (SOFA) score (≤ 11 vs. >11). All healthcare providers and patients were unaware of treatment assignment; only cell-

21 production–unit staff members were not blinded.

Production of the UC-MSCs-based advanced therapy medicinal product

The study treatments (UC-MSCs or placebo) were prepared by the Cell Therapy Unit and the
 MEARY Cell and Gene Therapy Center, which are two adjacent buildings, located in the
same hospital (Saint Louis Hospital, Paris, France). Briefly, the investigational advanced therapy medicinal product was a suspension of allogenic UC-MSCs, isolated from human UC Wharton's jelly by enzymatic digestion or the explant method, and amplified in vitro. Quality controls (Additional Table 1, 2 and 3) included viability, identity, purity, functionality (clonogenicity, immunosuppressive effects) and safety (microbiological, endotoxin and mycoplasma assays; karyotype).

Procedures

Each patient received three intravenous infusions of 10⁶ UC-MSCs/kg (maximum dose set at
80×10⁶ cells per infusion) or placebo on D1, D3±1 and D5±1. All patients were monitored for
any changes of pre-specified respiratory or cardiovascular parameters (*see* Additional file 1)
and were ventilated according to the modified ARDS Network lower tidal volume protocol.
Management of ARDS, septic shock and other organ failures followed international
guidelines [32,33].

Clinical and biological outcomes

The primary endpoint of the study was respiratory improvement assessed as the partial pressure of oxygen to fractional inspired oxygen (PaO₂/FiO₂)-ratio change between baseline (D0) and D7 post-randomization. Secondary and safety endpoints are provided in the Additional file 1. To assess UC-MSCs biological activity, biomarkers of endothelial, alveolar epithelial injury and inflammation were measured in plasma obtained on D0, D2, D4, D7, and D14, as exploratory endpoints. SARS-CoV-2 nucleocapsid antigenemia (N-antigenemia) and viral RNA levels were measured in plasma on D1 and D7. The level of donor-specific anti-human leucocyte antigen (HLA) antibodies (DSAs) directed against UC-MSCs was also measured on D0 and D14 to detect allo-immunization.

Statistical analyses

Continuous data are expressed as mean ± standard deviation or median [interquartile range, IQR]. Categorical parameters are expressed as numbers (percentages).

5 The primary endpoint was the evolution of the PaO₂/FiO₂ ratio between D0 and D7.
6 PaO₂/FiO₂ ratios of patients who died were imputed to 50, considered a minimum possible

7 value. Given the lack of literature data available at the time, the last observation carried

8 forward (LOCF) plus 10% imputation for the missing PaO₂/FiO₂ ratios of patients who

9 improved and were discharged from the ICU seemed reasonable and consistent with their

10 clinically observed recovery dynamics. A sensitivity analysis was computed using a LOCF

11 approach. The mean difference between the two groups was compared using analysis of

12 covariance (ANCOVA), adjusting for the D0 PaO₂/FiO₂ ratio and stratification factors.

Wilcoxon rank-sum test was used to analyse robustness. Statistical analyses used a two-sided
5% threshold of significance.

15 The principal analysis was conducted according to modified intent-to-treat, including all 16 randomized patients, except those who received no dose of the assigned treatment. A per-17 protocol analysis was also conducted on patients who had received the three planned doses of 18 the assigned treatment.

The evolutions of the criteria of interest were analysed at different times. Simple comparisons used Student's, Wilcoxon's, χ², or Fisher's exact tests according to the type of data. The evolution of clinical criteria, especially respiratory, was subjected to longitudinal modelling. Random-effects models were used to take into account a subject's repeated measurements. Biomarkers were log₁₀-transformed before analysis. Left-censoring (values below the limit of quantification) was accounted for either by modelling or imputation using each biomarker's half-value of the limit of quantification. In light of the exploratory nature of the study, no

penalty for the multiplicity of comparisons was implemented, except for cytokines that were
 subjected to Benjamini–Hochberg correction.

The sample size was chosen pragmatically in consideration of the capacity to produce UC-MSCs during a period when the need was urgent. However, the simulation run, which was meant to be only illustrative, suggested that the study should be able to demonstrate a 50% PaO₂/FiO₂-ratio increase from D0 to D7, based on the first information available on the PaO₂/FiO₂-ratio distributions of COVID-19 patients in ICUs.

All analyses and calculations were computed using R software version 4.0.3, R Core Team (2020), R Foundation for Statistical Computing, Vienna, Austria.

RESULTS

Patients

Patients were enrolled from April 6, 2020, to October 29, 2020, in the ICUs at ten study sites. Among the 107 patients screened for eligibility, 47 were randomly assigned to a treatment group and 45 received either UC-MSCs (n=21) or placebo (n=24; Figure 1). Their demographic characteristics are reported in Table 2. ARDS was mild, moderate or severe, respectively, in 31.1%, 48.9% and 20% of the patients. While SOFA and lung injury scores, and PaO₂/FiO₂ ratio were similar for the two groups, more placebo-group patients were on invasive mechanical ventilation, receiving vasopressors and neuromuscular blocking agents. Fifteen (71.4%) and 19 (79.2%) patients in the UC-MSC and placebo groups, respectively, received corticosteroids during the first 7 days (Table 3). During the first 28 days, the two groups had similar respiratory characteristics (Additional Table 4).

Treatment

During the 5-day treatment period, 81.0%, 9.5% and 9.5% of UC-MSC recipients received

three, two and one cell infusions, respectively (Table 3); they received a mean of $0.9\pm0.1\times10^{6}$ UC-MSCs/kg per dose (range $0.6-1\times10^{6}$ UC-MSCs/kg) (Additional Table 5). Two (4%) UC-MSC batches did not meet specifications because of insufficient cell counts (< $1.00\pm0.1\times10^{6}$ /kg) (Additional Table 5). Cell viability was $78.4\pm5.3\%$ and consistent across all batches ($\geq70\%$) (Additional table 2 and 3). UC-MSCs expressed CD90 (99.2±1.6%), CD73 (99.9±0.1%), and CD105 (97.0±1.9%), while CD45, CD34, CD11b, CD19, and HLA-DR ($0.8\pm0.7\%$) were below their defined positivity thresholds (2%). The colony-forming unit–fibroblast assay yielded a frequency of $1.8\pm1.1\%$ and all batches satisfied the >1% specification. Mixed lymphocyte-reaction assays, run to confirm UC-MSC immunomodulatory properties, showed significant dose-dependent inhibition of T-cell proliferation, with a peak inhibitory rate of $86\pm5\%$ for a UC-MSC/peripheral blood mononuclear cell ratio of 1:1.

Primary Endpoint

Of the 21 and 24 patients randomized to the UC-MSC and placebo groups, respectively, 17 and 18 of them had D7 primary endpoint measurements available. The respective numbers of patients whose D7 PaO₂/FiO₂-ratio values had to be imputed were four (three discharged and one died) and six (five discharged and one died). The primary outcome measure (PaO₂/FiO₂-ratio change between D0 and D7 post-randomization) did not differ significantly between the UC-MSC and placebo groups (median imputed values: 54.3 [IQR -15.5 to 93.3] vs 25.3 [IQR -33.3 to 104.6], respectively; ANCOVA treatment-effect estimate 7.4, 95% CI -44.7 to 59.7; P=0.77, Table 4).

Sensitivity, per-protocol, and subgroup analyses (Additional Table 6) showed similar results.
Although the D0 PaO₂/FiO₂ ratios for the two groups were similar (Figure 2A), the UC-MSC
group's ratio increased significantly from D0 to D7 (156·2±68·2 vs 188·3±74·2, respectively;

6 Secondary Endpoints

No significant between-group secondary-endpoint differences were observed for SOFA
scores, PaO₂/FiO₂ ratios, compliance, driving pressure change between D0 and D7 or D14,
organ-failure–free days, ventilation-free days, duration of ventilation, time to weaning, time
to ICU discharge, time to reach PaO₂/FiO₂ >200 or >300, and mortality to D28 (Table 4 and
Additional Figure 1).

Before and after D14, the numbers of adverse events, serious adverse events, and patients with any adverse and/or any serious adverse events were similar for the two groups (Table 5, Additional Table 7). In total, 36 (80%) patients experienced adverse events prior to D14, and 18 (40%) thereafter. The vast majority of adverse events were considered to be related to COVID-19 progression. Only one UC-MSC-group patient experienced an adverse event (diarrhoea) deemed possibly related to the study treatment. Only one placebo patient suffered a pre-specified adverse hemodynamic event within 6 hours of infusion onset (Additional Table 8). Six (28.6%) UC-MSC recipients had pre-formed DSA, i.e., present before treatment and directed against the HLA of the infused UC-MSCs received. On D14, three (14.3%) patients had synthesised de novo low-level DSA. Eleven (45.8%) placebo recipients had preformed anti-HLA, and no patient developed de novo significant anti-HLA immunization between D0 and D14.

Inflammatory biomarker-analysis (Additional Figures 2 and 3) and plasma virus-load results
(Additional Figure 4) are reported in Additional File 1. Later decreases of inflammatory

markers in the UC-MSC-treated group were the only significant difference found (Additional Figure 2).

DISCUSSION

The main finding of this study is that repeated intravenous infusions of UC-MSCs into patients with SARS–CoV-2-induced early ARDS were safe, but did not improve oxygenation, as reflected by the PaO₂/FiO₂-ratio change between D0 and D7, compared to the placebo group.

Two randomized trials have previously tested UC-MSCs in COVID-19 patients [30]. Whereas the study by Lanzoni et al. [30] included patients with ARDS and/or hypoxemia with SpO2 <94%, the patients enrolled by Dilogo et al. [31] were those with leukopenia and severe COVID-19 pneumonia, without further specification. Although the percentage of patients on invasive mechanical ventilation at inclusion was given in one study [30], neither of the two studies reported the distribution of invasively ventilated patients and their differences in UC-MSC and placebo groups, either at baseline or during follow-up. While the majority of patients described by Lanzoni et al. were also receiving adjuvant treatments administered at inclusion (i.e., remdesivir, convalescent plasma, tocilizumab, corticosteroids or hydroxychloroquine), this information was not given in the second study [31]. Finally, while the MSC-administration scheme consisted of two infusions of 100 million cells at day D0 and D3 for the former study, the latter administered one million/kg in a single infusion given between D2 and D30. The number of cell passages before use was not indicated in either study.

Our double-blind, randomized, placebo-controlled trial has several other key strengths, including serial determinations of a wide array of inflammation and immunity-related biomarkers at several times until D14, monitoring of allo-immunisation, and thorough

characterisation of the final UC-MSC–based product. Instead of assessing the three-lineage differentiation of our cells, we rather used a potency assay more specific for their intended immunomodulatory effect and to this end performed mixed lymphocyte reactions which confirmed their inhibitory effect on allogeneic T lymphocytes [34]. A final key strength is the multicenter design. However, the small number of patients included in ten centers over 6 months has several origins: 1) the pandemic dynamics were in successive waves, with periods of acceleration and deceleration of patient flows; 2) many patients admitted to the ICU were already included in phase 2 or 3 therapeutic trials, precluding inclusion in our trial; 3) some patients could not be included because of the unavailability of the manufacturing cell therapy unit. For the future, scaling up MSC production made possible by current technologies should allow to cover the needs of large numbers of patients.

A second important finding is that repeated UC-MSC infusions were not associated with any serious adverse events during treatment or thereafter (until D28). More specifically, data collected from continuous hemodynamic and respiratory monitoring during intravenous infusion of UC-MSC suggested good clinical tolerance during UC-MSC administration, with no transfusion incompatibility or infusion-related events. MSCs have already been used to treat a wide variety of diseases without safety issues, but those results might not be directly applicable to COVID-19, because one of the disease's hallmark is the potential for pulmonary thrombotic microangiopathy, which could have been worsened by UC-MSCs obstructing the pulmonary capillary bed, thereby causing right heart failure. No such event was documented in our trial. At least three factors might have contributed to the good tolerance of UC-MSC infusions: 1) when MSCs are cultured in 5% human-platelet lysate and for no longer than 3-4 passages, as in our trial, the reported cell size (in suspension) had been $\sim 17 \,\mu\text{m}$, which should keep cells within the safety range in terms of risk of vascular obstruction [35]; 2) the intrinsic prothrombotic activity of UC-MSCs is mitigated by intermediate-level anticoagulation

administration, now recommended and widely given to hospitalised COVID-19 patients [2], and it is reassuring that sCD40L levels, known to increase coagulation, were not elevated in UC-MSC-treated patients compared with controls (data not shown); and 3) the rapid apoptotic fragmentation of intravenously delivered UC-MSCs that became trapped in lung tissue and underwent progressive size diminution [36], and subsequent phagocytosis of apoptotic fragments by monocytes and neutrophils [37] could have contributed further to cell clearance from the vascular bed. Finally, it is noteworthy that only three of the UC-MSCtreated patients developed allo-immunisation to the infused cells and their mean fluorescence intensity (MFI) values were in the low range, unlikely to be cell-damaging [38]. That finding is consistent with previously reported clinical study results documenting the low immunogenicity of UC-MSCs [39].

Concerning efficacy, the trial did not meet its primary endpoint, since PaO₂/FiO₂-ratio changes between D0 and D7 did not differ significantly between UC-MSC- and placebo-infused patients. First, this lack of difference might suggest that, when COVID-19-associated respiratory failure is severe enough to warrant invasive mechanical ventilation or ventilatory support, the extent of lung damage outweighs the effects of UC-MSCs, at least when delivered according to the dosing schedule used herein, and thus their capacity to promote lung-tissue repair translating into improved oxygenation patterns. That lack of efficacy contrasts with the spectacularly improved survival obtained in two previous trials [30,31] that applied a similar double-blind, randomized design. However, in those studies, control-group mortality was unusually high (50% [31] to 58% [30] vs 21.9% herein), which allowed room for clearer demonstration of treatment effects. Furthermore, in both studies, although invasively ventilated patients were included, their distributions in each group were not specified; severity scores were missing and the timing of UC-MSC administration in relation to the ARDS stage (early or late) was either focused on the early [30] or the resolution phase

(i.e., >7 days) of ARDS [31], thereby making comparisons between the studies difficult. Second, the presence of pre-formed and *de novo* DSA in six (28.6%) and three (14.3%) UC-MSC-treated patients, respectively, might have altered therapeutic efficacy by accelerating MSC clearance, but this is unlikely because these patients' MFI levels were low-to-moderate and, except for one, remained below the 5,000 threshold level beyond which DSA-HLA-1 MFIs correlate with complement-dependent and antibody-dependent cell-mediated cytotoxicities [40]. The choice of the primary endpoint is a third possible explanation. The absence of FiO₂ or positioning (supine or prone) standardization for measuring PaO₂/FiO₂ might have increased the variability of its values from D0 to D7 [41], thereby hindering a beneficial or deleterious effect evaluated in the study. Moreover, some authors have questioned the prognostic value of PaO₂/FiO₂ in ARDS patients [42]. The PaO₂/FiO₂ ratio has several limitations [43]: 1) it does not reflect the ventilation applied and does not account for mean airway pressure or positive end-expiratory pressure; 2) it is dependent on barometric pressure; 3) it cannot distinguish hypoxemia due to alveolar hypoventilation from other causes, such as ventilation-perfusion mismatch and shunt; 4) it is markedly dependent on FiO₂; 5) it is highly dependent on oxygen extraction capacity; 6) it does not indicate oxygen content of the blood, or oxygen delivery to tissues. On the other hand, the study was designed at the very beginning of the first French pandemic wave, in February 2020, and, at that time, locally available data (from several ICUs in Paris) suggested a potentially notable improvement of survivors' gas exchange on D7 (no published or quantified data available at that time). Therefore, we decided to use the day-7 PaO₂/FiO₂ ratio as the primary endpoint. Another hypothesis explaining the absence of therapeutic effects is a potential lack of power of the study. Indeed, most phase 2 studies on ARDS had wide confidence intervals that, most often, limited generalisability [44]. Thus, our finding that the UC-MSC–group's—but not the placebo group's-PaO₂/FiO₂ ratio rose significantly from D0 to D7 might have reflected

some treatment efficacy that remained too modest to emerge with our sample size. However, this observation must be balanced against the fact that: 1) the numerical increase of the UC-MSC group's–PaO₂/FiO₂ ratio from D0 to D7 was no longer significant following the postimputation analysis, and 2) more placebo-group patients were invasively ventilated, under vasopressors and neuromuscular blocking agents, thereby indicating greater disease severity and potentially favoring the treated group.

Concerning circulating biomarker-level changes, it remains unclear whether the UC-MSC group's later appearance of the proinflammatory cytokine- and chemokine-level declines is a signature of the treatment, potentially leading to any benefit in terms of inflammation resolution. Results of previous randomized-controlled studies showed either a trend [31] or a significant immunoregulatory effect [30], with UC-MSC-treated patients having marked reductions of several circulating inflammatory biomarkers from D0 to D6–D7. Those heterogeneous findings can probably be explained by the differences in therapeutic doses, timing, choices of biomarkers to be measured and the small numbers of patients included, but also by the fact that those marker levels were determined in plasma and not the alveolar compartment. Indeed, Wick et al. recently found the MSC immunomodulatory effect in non-COVID-19 patients with ARDS to be more detectable in bronchoalveolar lavage at 48 hours post-MSC administration than in blood [45]. In future studies exploring the immunomodulatory impact of MSCs in patients with COVID-19-associated ARDS, it will probably also be important to measure these markers at early time points post-infusion and in bronchoalveolar lavage, if feasible.

We acknowledge that the STROMA–CoV-2 trial has limitations. They include the small sample size and the lack of a robust sample-size calculation explained by the paucity of published data at that time on the distribution and kinetics of PaO₂/FiO₂ ratio in this patient population; the likely patient-management modifications clearly illustrated by an almost

systematic use of corticosteroids during the second wave of the pandemic, with the caveat that, although in vitro study results indicated a generic cytotoxic effect of these drugs on MSCs, dexamethasone, which has been the gold standard during the pandemic (and was used in our trial), is the one with the least harmful effects on cell viability [46]; the higher percentage of invasively ventilated placebo-group patients on neuromuscular blocking agents and vasopressors, probably explained in part by the randomization-ratio change introduced between the 1st and 2nd pandemic waves, may have favored the treated group. However, the inclusion SOFA score, lung injury score, and PaO₂/FiO₂ ratio were comparable for the two groups; potential differences might exist in the bioactivities of the infused cells, despite quality-control consistency between the first and second batches, derived from 2 distinct donors; and the dosing and timing of delivery schedule are still debated. Indeed, the UC-MSC doses used have varied across studies, with a median dose of 100 million for intravenous delivery and a minimal effective dose ranging from 70 to 190 million/patient/dose [47]. Furthermore, although we are not aware of any comparison of a total aggregate dose given as a single bolus vs its fractionation over time, the rapid UC-MSC clearance led us to adopt repeated dosing, in hopes of inducing a longer-term effect. Those considerations rationalized our choice of three 1×10^6 UC-MSCs/kg/dose repeated every other day, a dosing strategy consistent with several of the registered cell-therapy trials on COVID-19 patients [48]. However, it remains to be assessed whether outcomes can be improved by higher doses and/or longer time intervals between cell deliveries.

22 Conclusions

The results of this phase 2b, multicentre, double-blind, randomized, placebo-controlled trial
showed no efficacy of human UC-MSCs on the PaO₂/FiO₂-ratio change between D0 and D7
in patients with SARS–CoV-2-induced ARDS compared to placebo. D28 mortality also did

not differ. Despite the lack of statistically significant differences, UC-MSC-treated patients' greater PaO₂/FiO₂-ratio increase between D0 and D7, compared to placebo-infused controls, might represent a signal warranting further investigation on a larger patient population. Repeated UC-MSC infusions were not associated with any serious adverse events during treatment or thereafter (until D28). Consequently, to better assess in which direction this treatment strategy shifts the risk-benefit and cost-effectiveness balances, pursuit of this avenue of research on COVID-19-associated pneumonia would be notably enhanced by greater homogeneity of patient demographics and standardized therapeutic protocols, reappraisal of the most clinically relevant endpoints and larger sample sizes.

List of abbreviations

ANCOVA analysis of covariance, ARDS acute respiratory distress syndrome, CFU colony-forming unit, COVID-19 coronavirus disease-2019, D day, DSAs donor-specific anti-human leukocyte antigen antibodies, PaO_2/FiO_2 partial pressure of oxygen to fractional inspired oxygen, HLA human leukocytes antigen, ICU intensive care unit, IL interleukin, IQR interquartile range, LOCF Last Observation Carried Forward, MFI mean fluorescence intensity, MSCs mesenchymal stromal cells,; RT-PCR reverse transcriptase-polymerase chain reaction, SARS-COV-2 severe acute respiratory syndrome coronavirus-2, SOFA Sequential Organ-Failure Assessment, UC umbilical cord.

Declarations

Ethics approval and consent to participate

The National Review Board of Île-de-France III approved the trial (CNRIPH 20.03.26.39722) that was authorized by the French National Agency for Medicines and Health Products Safety (EudraCT 2020-001287-28). Written informed consent was obtained from patients or a le-gally designated representative.

Consent for publication

Not applicable

Availability of data and materials

Qualified clinical researchers can request access to de-identified participant dataset, informed consent forms and related documents, including the study protocol that underlie this article through submission of a proposal with a valuable research question to the corresponding author, subject to agreement of a contract.

Competing interests

AD declared grants or contracts from Philips, Fisher & Paykel; French Ministry of Health; Respinor; Lungspacer; consulting fees from Lungspacer, Respinor; payments or honoraria for lectures, presentations, speaker bureaus, manuscript writing or educational events from Fisher & Paykel, Getinge, Lungspacer, Gilead, Lowenstein, Astra; support for attending meetings and/or travel from Fisher & Paykel, Lungspacer; received equipment, materials, drugs, medical writing, gifts or other services from Lungspacer, Respinor. MF declared grants or contracts from BioMérieux and MSD; French Ministry of Health; consulting fees from Pfizer; payments or honoraria for lectures, presentations, speaker bureaus, manuscript writing or

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Authors' contributions

AM, PM, JL, and JR contributed to overall study design and developed the protocol. AM, CH, JM, ONT, JLD, AD, NH, DA, CM, SD, EW, CP, GV, MF, JMC, BM, JES, and GP were responsible for study enrolment, data collection, and manuscript editing. MM, AC, HB, CM, GC, and JL were responsible for manufacturing the experimental drug and preparing the placebo. AM, **ONT**, MR, PM, AC, and MM were responsible for biorepository management, biomarker analyses, and interpretation. NT was responsible for statistical analyses of biomarker data. JR and MHD were responsible for statistical analyses of clinical data. AM, PM, JR, AC, MM, SM, SB, MR, ONT, and JL contributed to the data analysis and manuscript writing. All authors revised the report and read and approved the final version before submission.

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FIGURE LEGENDS

Figure 1. Flow chart of the trial.

ARDS acute respiratory distress syndrome. ATMP advanced therapeutic medicinal product. D day. ELS extracorporeal life support. ICU intensive care unit. UC-MSCs umbilical cordderived mesenchymal stromal cells.

Figure 2. Primary endpoint: PaO₂/FiO₂ values and their changes between days 0 and 7.

(A) Baseline (D0) and D7 PaO₂/FiO₂ ratios were similar for the two groups. Box plots of

PaO₂/FiO₂ ratios: internal *horizontal lines* are the medians; *lower* and *upper box limits* are the

25th-75th interquartile range, respectively; and vertical bars represent the 10th and 90th

percentiles. (B) PaO₂/FiO₂ ratios increased significantly from D0 to D7 in the UC-MSC group

(respectively, $156 \cdot 2 \pm 68 \cdot 2 vs$ $188 \cdot 3 \pm 74 \cdot 2$; Wilcoxon signed-rank exact test). The placebo

group's PaO₂/FiO₂ ratios on D0 and D7 were comparable (respectively, 171·2±72·9 vs

169.8±85.6, Wilcoxon signed-rank exact test). UC-MSCs group in *red*; placebo group in *blue*.

D day. PaO₂/FiO₂ ratio of partial pressure of oxygen to fractional inspired oxygen. UC-MSCs

umbilical cord-derived mesenchymal stromal cells.

1 Table 1: Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Age>18 years	Age <18 years
Rreverse transcriptase-polymerase chain reaction -con-	Acute respiratory distress syndrome present for >96
firmed SARS–CoV-2 infection	hours
Berlin criteria-defined acute respiratory distress syndrome	Pulmonary fibrosis
for <96 hours	Pulmonary hypertension (WHO classification class III
Respiratory support (invasive or non-invasive mechanical	or IV)
ventilation, and/or high-flow nasal oxygenation) with	Pulmonary embolism within the previous 3 months
positive end-expiratory pressure equivalent ≥5 cm H ₂ O	Extracorporeal membrane oxygenation or life support
	Immunocompromised status including use of immu-
	nosuppressive medications
	Pregnancy or breastfeeding
	Treatment for cancer in the past 2 years
	Underlying medical condition with life expectancy <6
	months
	Moderate-to-severe liver disease (Child–Pugh score
	>12)
	Severe chronic lung disease with the use of home
	oxygen and/or partial arterial pressure of carbon
	dioxide >50 mm Hg
	Patients not committed to full support (ie, had do not
	resuscitate or limit life support orders)
	Participation in another trial of COVID-19 therapeu-
	tics

Table 2: Patients' baseline characteristics

	UC-MSC (n=21)	Placebo (n=24)	p value
Age, years	64 (10·4)	63·2 (11·4)	0.82
Male sex	17 (81%)	20 (83·3%)	1
Body mass index	28.6 (3.5)	28 (5.5)	0.68
Obesity	7 (33·3%)	6 (25%)	0.54
Sepsis-related Organ-Failure Assessment score	5.5 (2.7)	5.9 (2.7)	0.64
Mean arterial pressure, mm Hg	91.3 (18.3)	81.5 (16.9)	0.07
On vasopressors	5 (23.8%)	14 (58·3%)	0.02
Comorbidities			
Chronic obstructive pulmonary disease	0 (0%)	1/15 (6·7%)	1
Active smoking	0 (0%)	0 (0%)	0.24
Chronic heart failure	0 (0%)	0 (0%)	1
Atrial fibrillation	2/15 (13·3%)	0 (0%)	0.21
Hypertension	11/15 (73·3%)	10/15 (66·7%)	0.47
Coronary artery disease	2/15 (13·3%)	2/15 (13·3%)	1
Stroke	2/15 (13·3%)	1/15 (6·7%)	0.59
Immunodeficiency	0 (0%)	0 (0%)	1
Active neoplasia	0 (0%)	0 (0%)	1
Chronic corticosteroid intake	0 (0%)	0 (0%)	1
Immunomodulatory drugs	2/17 (11.8%)	0 (0%)	0.2
Respiratory characteristics			
Ventilatory support (NIV and/or HFNO)	10 (47·6%)	4 (16·7%)	0.02
Invasive mechanical ventilation	11 (52·4%)	20 (83·3%)	0.02
Tidal volume, mL/kg PBW	6·2 (0·7, n = 11)	6·3 (0·8, n = 20)	0.72
Plateau airway pressure, cm H ₂ O	21·8 (4·2, n = 10)	24·8 (5·1, n = 17)	0.12
PEEP	10·8 (2·9, n = 11)	11·2 (3·2, n = 20)	0.72
Driving pressure	11·3 (4·3, n = 10)	13·2 (3·9, n = 17)	0.25
Compliance, mL/cm H ₂ O	45·2 (27·8, n = 10)	35·2 (14·9, n = 17)	0.29
SpO ₂ , %	94.6 (3.4)	96·0 (3·0, n = 23)	0.16
PaO ₂ /FiO ₂ , mm Hg	156·2 (68·2)	171.2 (72.9)	0.53
Lung injury score	3.0 (0.7)	2.8 (0.5)	0.61
PaCO ₂ , mm Hg	40 (8·5)	43.2 (9.8)	0.17
рН	7.41 (0.1)	7.37 (0.1)	0.27
Neuromuscular blocking agents	6 (28·6%)	16 (66·7%)	0.01
Ventilation mode			
Volume control	11/11 (100%)	19/20 (95%)	1
Pressure control	0 (0%)	0 (0%)	1
Pressure support	0 (0%)	1/20 (5%)	1

Values are expressed as mean (standard deviation) or number (%). Information was available for all patients, unless indicated otherwise. *HFNO* high-flow nasal oxygen therapy. *NIV* non-invasive ventilation. *PaCO*₂ partial pressure of arterial carbon dioxide. *PaO*₂/*FiO*₂ ratio of partial pressure of oxygen to fractional inspired oxygen. *PBW* predicted body weight. *PEEP* positive end-expiratory pressure. *SpO*₂ peripheral capillary oxygen saturation. *UC-MSCs* umbilical cord-derived mesenchymal stromal cells.

Table 3: Assigned treatment doses received and corticosteroid administration from day 0 to day 7.

	UC-MSCs (n=21)	Placebo (n=24)	
Number of doses received over 7 days	2.7 (0.6)	2.7 (0.5)	
One	2 (9.5%)	1 (4·2%)	
Тwo	2 (9.5%)	4 (16·7%)	
Three	17 (81%)	19 (79·2%)	
Corticosteroids administered for 7 days	15 (71·4%)	19 (79·2%)	
Values are expressed as number (%). UC-MSCs umbilical cord-derived mesenchymal stromal cells.			

Table 4: Main clinical outcomes

	UC-MSCs (n=21)	Placebo (n=24)	Estimate (95% CI)	p value
Primary endpoint				
PaO ₂ /FiO ₂ -ratio change D0–D7	54.3 [–15.5; 93.3]	25.3 [–33.3; 104.6]	7.4 (-44.7; 59.7)	0.77
(principal analysis)				
PaO ₂ /FiO ₂ -ratio change D0–D7	54.3 [–15.5; 93.3]	25.3 [–33.3; 83.1]	12.5 (-33.8; 56.7)	0.59
(sensitivity analysis)				
Secondary endpoints			Median difference	
			(95% CI),	
			HR or sub-HR	
PaO ₂ /FiO ₂ -ratio change D0–D14	11.0 [–39; 72.7]	28.2 [–1; 67.3]	1.69 (-82.1; 90.7)	1
Ventilation-free days to D28, n	17.0 [0; 25.0]	12.0 [0; 19.7]	0.5 (-3.0; 8.0)	0.61
Ventilation duration to D28, n	9.0 [3.0; 20.0]	10 [5.7; 20.0]	-2.5 (-8.0; 3.0)	0.38
Ventilation duration to D28 for	11.0 [6.0; 24.0]	13.0 [7.0; 22.0]	-0.5 (-8; 6)	0.79
recipients of 3 UC-MSC doses				
SOFA-score change D0–D7	–1.5 [–2; 0.75]	-2 [-3.2; 0.2]	0.5 (-2.0; 3.0)	0.60
SOFA-score change D0–D14	–0.5 [–1.2; 1.0]	-3.0 [-3; -1.0]	1.5 (–1.0; 5.0)	0.12
Organ-failure–free days to D14, n	3.0 [0; 6.0]	2.0 [0; 9.0]	-0.5 (-4.0; 3.0)	0.96
Organ-failure–free days to D28, n	16.0 [2.0; 20.0]	15.0 [0.75; 23]	-0.5 (-7.0; 4.0)	0.68
Days to reach PaO ₂ /FiO ₂ >200	5.0 [0; 16.0]	2.5 [0; 6.5]	0.74 [0.3; 1.6]	0.44
Days to reach PaO ₂ /FiO ₂ >300	12.0 [7.0; 23.0]	15.0 [5.0; 27.0]	1.1 [0.5; 2.3]	0.87
Days to ICU discharge*	15.0 [8.0; NA]	13.0 [5.5; 27]	0.8 [0.4; 1.7]	0.59
Days to weaning**	13.0 [9.0; NA]	17.0 [8.0; NA]	1.3 [0.5; 3.3]	0.55
Compliance change D0–D7	–3.6 [–11.8; 4.7]	-0.1 [-4.5; 2.4]	0.4 (-24.7; 25.5)	1
Compliance change D0–D14	-3.0 [-3.0; -3.0]	2.5 [–0.9; 11.7]	-5.52 (-35.4; 1.1)	0.8
Driving pressure change D0–D7	0.5 [-3.2; 4.2]	0.5 [–1.2; 2.2]	0 (–13.0; 13.0)	1
Driving pressure change D0–D14	1.0 [1.0; 1.0]	–1.5 [–2.0; 0.2]	2.5 (-3.0; 3.0)	0.8
D28 mortality	5.0 (26.3%)	4.0 (18.2%)	2.0 (0.5; 8.5)	0.36

Values are expressed as median [interquartile range], or number (%), unless stated otherwise. *CI* confidence interval. *D* day. *HR* hazard ratio. *ICU* intensive care unit. *NA* not applicable. *PaO*₂/*FiO*₂ ratio of partial pressure of oxygen to fractional inspired oxygen. *PEEP* positive end-expiratory pressure. *SOFA* Sepsis-related Organ-Failure Assessment. *UC-MSCs* umbilical cord-derived mesenchymal stromal cells.

*Censored at day of death for patients having died before D28 and censored at D28 for those patients still in the ICU at D28

**Estimated for the subgroup of patients ventilated at randomization, i.e. 31 patients (11 in CSM-CO group and 20 in placebo group). At Day 28, 6 had died (and censored at time of death), 17 were weaned, and 8 were alive and not weaned yet (censored at D28).

	UC-MSC	Placebo	Total	p value
Adverse events D0–D14				
Subjects with AEs	18/21 (85·7%)	18/24 (75%)	36/45 (80%)	0.47
AEs reported	49/97 (50·5%)	48/97 (49·5%)	97 (100%)	0.29
Subjects with SAEs	6/21 (28·6%)	6/24 (25%)	12/45 (26.7%)	0.79
SAEs reported	10/49 (20·4%)	6/48 (12·5%)	16/97 (16·5%)	0.29
AEs by severity				0.99
Mild	16/49 (32·7%)	15/48 (31·3%)	31/97 (32%)	
Moderate	24/49 (49%)	24/48 (50%)	48/97 (49.5)	
Severe	9/49 (18·4%)	9/48 (18·7%)	18/97 (18·6)	
AE grade*				0.14
Grade 1	19/47(40·4%)	10/48 (20.8%)	29/95 (30.5%)	
Grade 2	15/47 (31·9%)	19/48 (39·6%)	34/95 (35.8%)	
Grade 3	9/47 (19·1%)	16/48 (33·3%)	25/95 (26·3%)	
Grade 4	4/47 (12·5%)	3/48 (6·3%)	7/95 (7·4%)	
AEs by treatment relatedness				0·41
Possible	1/48 (2·1%)†	0 (0%)	1/95 (1.1%)	
Other treatment	4/48 (8·3%)	2/47 (4·3%)	6/95 (6·3%)	
Other disease	1/48 (2·1%)	1/47 (2·1%)	2/95 (2·1%)	
COVID-19 progression	28/48 (58·3%)	37/47 (78·7%)	65/95 (68·4%)	
Other causes	3/48 (6·3%)	2/47 (4·3%)	5/95 (5·3%)	
Undetermined	11/48 (22·9%)	5/47 (10.6%)	16/95 (16·8%)	
Adverse events after D14				
Subjects with AEs	9/21 (38·1%)	9/24 (37.5%)	18/45 (40%)	0.71
AEs reported	19/42 (45·2%)	23/42 (54·8%)	42 (100%)	1.00
Subjects with SAEs	4/21 (19%)	4/24 (16·7%)	8/45 (17·8%)	1.00
SAEs reported	4/19 (21·1%)	4/23 (17·4%)	8/42 (19%)	1.00
AEs by severity				0.19
Mild	7/19 (36·8%)	3/23 (13%)	10/42 (23.8%)	
Moderate	7/19 (36·8%)	12/23 (52·2%)	19/42 (45·2%)	
Severe	5/19 (26·3%)	8/23 (34.8%)	13/42 (31%)	
AE grade*				0·18
Grade 1	7/18 (38·9%)	3/23 (13%)	10/41 (24·4%)	
Grade 2	7/18 (38·9%)	15/23 (65·2%)	22/41 (53·7%)	
Grade 3	1/18 (5·6%)	3/23 (1%)	4/41 (9·8%)	
Grade 4	3/18 (16·7%)	2/23 (8.7%)	5/41 (12·2%)	
AEs by treatment relatedness				0.54
Possible	0 (0%)	0 (0%)	0 (0.0%)	
				1

1 Table 5: Summary of the 139 reported adverse events occurring in the 24 UC-MSC- and 21 placebo-treated patients

Other treatment	3/14 (21·4%)	4/20 (20%)	7/34 (20·6%)	
Other disease	1/14 (7·1%)	0 (0%)	1/34 (2·9%)	
COVID-19 progression	8/14 (57·1%)	11/20 (55%)	19/34 (55·9%)	
Other causes	2/14 (14·3%)	2/20 (10%)	4/34 (11·8%)	
Undetermined	0 (0%)	3/20 (15%)	3/34 (8.8%)	

Values are expressed as number (%). AEs adverse events. SAEs severe adverse events. UC-MSC umbilical cord-derived mesenchymal stromal cells.

*Grade from the Common Terminology Criteria for Adverse Events classification. *Possible non-serious treatment-related AE: diarrhoea.



FIGURE 2



UC-MSCs placebo

1 Treatment of COVID-19-associated ARDS with mesenchymal stromal cells: a

- 2 multicenter randomized double-blind trial.
- 3
- 4 Antoine Monsel
- 5 Caroline Hauw-Berlemont
- 6 Miryam Mebarki
- 7 Nicholas Heming
- 8 Julien Mayaux
- 9 Otriv Nguekap Tchoumba
- 10 Jean-Luc Dieh
- 11 Alexandre Demoule
- 12 Djillali Annane
- 13 Clémence Marois
- 14 Sophie Demeret
- 15 Emmanuel Weiss
- 16 Guillaume Voiriot
- 17 Muriel Fartoukh
- 18 Jean-Michel Constantin
- 19 Bruno Mégarbane
- 20 Gaëtan Plantefève
- 21 Stéphanie Mallard-Castagnet
- 22 Sonia Burrel
- 23 Michelle Rosenzwajg
- 24 Nicolas Tchitchek
- 25 Hélène Boucher-Pillet
- 26 Guillaume Churlaud
- 27 Audrey Cras
- 28 Camille Maheux
- 29 Chloé Pezzana
- 30 Mamadou Hassimiou Diallo
- 31 Jacques Ropers
- 32 Philippe Menasché
- 33 Jérôme Larghero
- 34 on behalf of the APHP STROMA–CoV-2 Collaborative Research Group

Additional File 1

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- 36 37
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1 METHODS

2 Patients and Exclusion Criteria

3 Eligible patients had Berlin criteria-defined ARDS for <96 hours, reverse transcriptasepolymerase chain reaction (RT-PCR)-confirmed SARS-CoV-2 infection, and were receiving 4 respiratory support (invasive or non-invasive mechanical ventilation, and/or high-flow nasal 5 6 oxygenation), with positive end-expiratory pressure (PEEP) equivalent >5 cm H2O. The need 7 for high-flow nasal oxygen therapy was sufficient to define "ventilatory support" status, regardless of the gas flow and FiO₂ parameters. Exclusion criteria included age <18 years, 8 acute respiratory distress syndrome (ARDS) present for >96 hours, pulmonary fibrosis, 9 10 pulmonary hypertension (WHO classification class III or IV), pulmonary embolism within the 11 previous 3 months, extracorporeal membrane oxygenation or life support, 12 immunocompromised status (i.e., constitutional like human immunodeficiency virus, or drug-13 induced with chronic intake of immunosuppressant(s) or corticosteroids at an immunosuppressive dose for >1 month (≥ 10 mg/day of prednisolone equivalent)), pregnancy 14 15 or breastfeeding, treatment for cancer in the past 2 years, an underlying medical condition 16 with life expectancy <6 months, moderate-to-severe liver disease (Child–Pugh score >12), 17 severe chronic lung disease with the use of home oxygen, or partial arterial pressure of carbon 18 dioxide >50 mm Hg. Patients not committed to full support (ie, had do not resuscitate or limit life support orders) were also excluded. Written informed consent was obtained from patients 19 20 or a legally designated representative. When the latter was absent, the investigator decided to 21 include the patient because of the therapeutic emergency. In that case, the patient's or 22 representative's consent for continuation of the protocol was to be obtained as soon as possible. 23

24

25 Randomization and Blinding

1 Randomization and data collection were centralised using a web-based electronic case-report 2 form (Cleanweb Telemedicine Technologies[™]) on a sponsor-operated secure server. 3 To maintain blinding of the investigators and clinicians, conditioning, labelling and distributions of UC-MSCs and placebo pouches were rigorously identical, with both being 4 5 pre-conditioned in opaque bags. The intravenous infusion tubing set was not blinded *per se*. 6 Only the bag was covered. However, it has been considered that, given the dilution of the cell 7 suspension, and, thus, the transparent appearance of the fluid, it was not possible for the 8 treating staff to ascertain whether or not the patient received the treatment or the placebo only 9 by looking at the infusion set. Investigators remained unaware of treatment allocations until 10 the database was fully cleansed and exported for statistical analysis.

11

Production and Characterisation of UC-MSC-based Advanced Therapy Medicinal Product (ATMP)

14 The experimental treatment is a suspension of allogenic mesenchymal stromal cells isolated from human umbilical cord Wharton's jelly (UC-MSCs). Two UCs were collected from two 15 healthy donors, according to the European Directive 2004/23/EC, after written informed 16 17 consents had been obtained and serological testing done according to national regulatory 18 agency requirements. At the end of the production process, the two cell populations were used 19 separately., *i.e.*, each patient received UC-MSC doses from a single umbilical cord. Full-term 20 delivery cords were collected and processed within 24 hours. The active substance was 21 produced at the Saint-Louis Hospital Cell Therapy Unit of Paris and the ATMP was produced 22 at the MEARY Cell and Gene Therapy Center, according to good manufacturing practices 23 (GMPs). Briefly, MSCs were isolated from UCs by enzymatic digestion or the explant 24 method, then expanded in complete culture medium composed of Nutristem® MSC XF Basal 25 Medium (Biological Industries, Ref 05-200-1A) + Nutristem® MSC XF Supplement Mix

1 (Biological Industries, Ref 05-200-1U) + 5% irradiated platelet lysate MultiPL'100i

(Macopharma, Ref BC0190032) + sodium heparin 2 IU/mL (Panpharma, Ref 5520508). Cells
were maintained at 37°C in a humidified atmosphere with 5% CO₂ and the culture medium
was changed twice a week. UC-MSCs were expanded until passage 4 maximum, harvested
using a Recombinant Trypsin-EDTA Solution (Biological Industries, Ref 03-079-1B), and
cryopreserved in a 10% dimethyl sulfoxide solution at a mean (±SD) dose of 100±10×10⁶
UC-MSCs per bag.

Quality controls of the experimental ATMP included cell concentration, identity, and purity 8 9 (immunophenotype), functionality (viability, clonogenicity, and immunosuppressive effects 10 assessed by mixed lymphocyte reaction [MLR]), and safety (microbiological sterility, 11 endotoxins, and mycoplasma assays with a posteriori results; karyotype). Cell number was 12 determined using the Nucleocounter NC 200® (ChemoMetec, Denmark). The UC-MSC 13 phenotype was determined by flow cytometry (MacsQuant10, Miltenyi), using the surface antigens CD105, CD73, CD90, CD45, CD34, CD11b, CD19, and HLA-DR from the BD 14 15 stemflow hMSC Analysis Kit (BD Biosciences, ref 562245). Cell viability was evaluated by using the eBioscience Fixable Viability Dye eFluor 780 assay (Invitrogen, ref 65-0865). Cell 16 17 clonogenicity was assessed using the colony-forming-unit-fibroblast (CFU-F) assay. 18 To assess UC-MSC immunomodulatory properties, ie, evaluate their inhibitory effect on Tcell proliferation in vitro, MLRs were run, as described by Nicotra et al (1). Our results 19 showed that UC-MSCs significantly inhibited T-cell proliferation by 86±5% for a UC-20 21 MSC/PBMC ratio of 1:1. Pertinently, that inhibitory effect was dose-dependent, decreasing 22 proportionally to the UC-MSC/PBMC ratio: (78±10% at ratio 1:3; 77±10% at ratio 1:10, 70±3% at ratio 1:30, 44±20% at ratio 1:100 and 18±15% at ratio 1:300). Microbiological 23 testing used aerobic and anaerobic BacT/ALERT® (BioMerieux, ref 259789/259790) in 24 25 BacTALERT® VIRTUO® Microbial Detection System (BioMerieux). Endotoxins were

quantified on Multiskan Sky Spectrophotometer (ThermoFisher Scientific) using Chromo LAL® reagents (Associates of Cape Cod, ref C0031) and mycoplasma on QuantStudio 5
 Real-Time PCR System (ThermoFisher Scientific) using Venor® GeM qEP Kit (Minerva
 Biolabs, ref 11-9250). The karyotype was determined before MSC cryopreservation in a
 certified laboratory (Cerba, France) on a minimum of 20 metaphases.

6 The day of patient treatment, one bag was thawed. The collected cells were washed and underwent a final quality control before being suspended at the dose of 10^6 cells/kg in a final 7 volume of 150 mL of 0.9% NaCl/0.5% albumin. The maximum dose was set at 80×10⁶ cells 8 9 per infusion for the following reasons: 1) fitting a reasonable theoretical average weight; 2) controlling the risk of pulmonary capillary trapping; 3) making the protocol compatible with 10 11 the capacity to produce UC-MSCs. Moreover, this dose was repeated 3 times over 5 days in 12 our protocol, making the maximum total dose was 240 million cells per patient, which 13 remains well above the median therapeutic dose of 100 million cells per patient described in 14 the literature. The final product was released according to the following criteria and specifications: 10^6 UC-MSCs/kg dose, viability \geq 70%, identity markers matching a typical 15 MSC phenotype. Finally, the pouch was transferred to the ICU at 18–24°C, within 4 hours 16 after thawing. The placebo was a solution of 150 mL of 0.9% NaCl. A single manufacturing 17 center (production and quality control) provided cells for all investigating centers. All the 18 19 thawing, washing and dose adjustment steps were done in a dedicated clean room at MEARY 20 Center (SLS – Saint Louis Hospital), with a Quality-Control Laboratory located on the same 21 floor of the building. Product-expiration time was determined from the final bag-sealing step 22 (the 4-hour period did not include the thawing/washing steps).

23 The experimental ATMP was not stored before administration. After release, the product was

- 24 immediately shipped to the pharmacist of the participating centers who brought it
- immediately to the ICU for administration. The bag was transported to centers at +18 to +24 $^{\circ}$
C in a sealed container by an approved carrier in an average time of 59 min (TNN: 55 min;
BJN: 63 min; PSL: 39 min; HEGP: 48 min; RPC: 74 min, ARG: 92 min; LRB: 42 min). The
infusion could start within 4 hours following ATMP-manufacturing completion (weighing
and final sealing of the bag), i.e., the infusion duration period was not included in this 4-hour
product expiration time.

6

7 Circuit of the UC-MSC-based ATMP

After its release, the experimental ATMP or the placebo was transferred to the pharmacy of
each recruiting center, with instructions for use and traceability documents. After receipt and
control, the pharmacy distributed the treatment pouches to the ICU. The experimental
ATMP/placebo was administered to the patient by slow intravenous perfusion, within 4 hours
after thawing. UC-MSCs or placebo treatments were infused intravenously by drip over 60
min through a standard blood-filter tubing set (pore size 170 µm).

14

15 Clinical and Biological Outcomes

16 Secondary endpoints were all-cause mortality at D28, number of ventilator-free days to D28, 17 duration of ventilation in patients alive on D28, number of intensive care-free days to D28, 18 number of organ-failure-free days to D28, SOFA score, lung injury score, driving pressure, 19 and respiratory lung compliance. Patients were considered weaned-off mechanical ventilation on the first day invasive mechanical ventilation was stopped, taken as the weaning date. The 20 21 "days-to-weaning" duration corresponds to the time between the start of invasive mechanical 22 ventilation and the first day without invasive mechanical ventilation. Safety endpoints were 23 the number of adverse events attributable to UC-MSC administration, the number of pre-24 specified infusion-related adverse events (listed below) during the infusion of study 25 treatments (ATMP or placebo), and the 6 hours following infusion onset, during the 24 hours

1	following each administration on D1, D3 (± 1 day) and D5 (± 1 day), during the treatment
2	period from D1 to D7, and any adverse events associated with UC-MSC infusion from D1 to
3	D28. Pre-specified infusion-related adverse events from D0 to D7 could be assessed by
4	continuous hemodynamic and respiratory monitoring (e.g., heart rate, blood pressure, oxygen
5	saturation). The incidence and nature of all serious adverse events were reviewed periodically
6	and independently assessed by the Data-Safety-Monitoring Board to determine whether they
7	were related to UC-MSC administration, with special focus on events that would be
8	unexpected in a critically-ill patient with ARDS.
9	
10	List of Prespecified Respiratory or Cardiovascular Infusion-Related Adverse Events
11	During the infusion of study treatments (ATMP or placebo):
12	• A clinical picture compatible with a transfusion incompatibility or an infusion-linked
13	event (urticaria, bronchospasm)
14	During the 6 hours following infusion onset of study treatments:
15	• Onset of hemodynamic instability requiring administration of noradrenaline $\geq 2 \text{ mg/h}$;
16	decreased blood pressure necessitating a higher dose of noradrenaline ≥ 2
17	mg/h compared to its initial value
18	• Ventricular tachycardia, ventricular fibrillation or cardiac arrest
19	• Development of severe hypoxia (partial pressure of oxygen to fractional inspired
20	oxygen (PaO ₂ /FiO ₂)) \leq 60 mm Hg or \geq 50% of its preinfusion value.
21	During the 24 hours following each infusion
22	• Any cardiac arrest.
23	During the treatment period from day (D) 1 to D7
24	• Massive pulmonary embolism with hemodynamic repercussions
25	• Deterioration of arterial oxygenation with severe hypoxia unexplained by the

evolution of the SARS-CoV-2-induced ARDS and requiring implantation of
 venovenous-extracorporeal membrane oxygenation
 Any clinical evidence identified by the physician as unusual by its characteristics,
 frequency, or severity compared to the clinical evolution of ARDS.

Analysis of Inflammatory Cytokines, Chemokines, Growth Factors and Biomarkers in Plasma at Baseline (D0) and D2, D4, D7, and D14 After Start of Infusions. The Luminex[®]-based multiplexed immunoassays with fluorescent microspheres (Milliplex[®]) MAP assays-HCP2MAG-62K-PX23, TFGBMAG-64K-03, and HCYTA-60K-PX48) were used to quantify the plasma concentrations of 48 cytokines, chemokines, and growth factors.

11 The epithelial and endothelial biomarkers keratinocyte growth factor (KGF), surfactant

12 protein D (SPD), angiopoietin (ANGP-1 and -2), receptor for advanced glycation end

13 products (RAGE) were measured using the following kits, respectively: Human KGF/FGF-7

14 Quantikine ELISA Kit#DKG00, Human SP-D Quantikine ELISA Kit#DSFPD0, Human

15 Angiopoietin-1 Quantikine ELISA Kit#DANG10, Human Angiopoietin-2 Quantikine ELISA

16 Kit#DANG20, and Human RAGE Quantikine ELISA Kit#DRG00 from R&D Systems®. All

17 the analytes were quantified, according to the manufacturer's protocol, with ELISA kits

18 (Quantikine ELISA Kits, Bio-Techne SAS, Rennes, France) and Luminex assays (Milliplex,

19 Millipore SAS, Sain-Quentin-en-Yvelines, France).

20

21 SARS-CoV-2 Nucleocapsid-Antigenemia (N-Antigenemia) Assay

22 Prior to analysis, plasma samples were stored at -80°C. N-Antigenemia levels were

23 determined with the coronavirus disease (COVID)-Quantigene® CE-IVD ELISA microplate

24 assay (AAZ, Boulogne-Billancourt, France) according to manufacturer's recommendations,

adapted to an ETI-MAX®3000 analyzer (DiaSorin, Gerenzano, Italy). Briefly, 50 µL per well

of plasma or standards consisting of recombinant SARS-CoV-2 N-Antigenemia were 1 2 deposited in a 96-well microplate coated with anti-SARS-CoV-2 N-Antigenemia antibodies; 3 50 µL of a solution containing a biotinylated–anti-SARS–CoV-2 N-Antigenemia antibodies were added and incubated at 37°C for 60 minutes; plates were washed with a phosphate 4 5 buffer solution, and 100 µL of a solution containing horseradish peroxidase-conjugated 6 streptavidin were added, followed by incubation for 30 minutes at 37°C; plates were washed with the phosphate buffer solution; 50 μ L of a solution containing the TMB (3,3',5,5'-7 8 tetramethylbenzidine) substrate and 50 µL of a second solution containing urea were added; 9 after 15 minutes at 37°C, the colorimetric reaction was stopped by adding 50 µL of sulfuric 10 acid (H₂SO₄). Absorbance values were measured at 450 nm, with a reference set at 630 nm. 11 The N-Antigenemia-positivity threshold was 2.97 pg/mL.

12

13 SARS-CoV-2 Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Viral RNA in plasma was detected with the Simplexa[™] COVID-19 Direct assay (DiaSorin
[DiaSorin SA, Antony, France]), targeting the viral *ORF1ab* and *S* (spike) genes, and
including an RNA internal control to detect RT-PCR failure and/or inhibition. Briefly, 50 µL
of sample and 50 µL of reaction mix were added to their specific wells on a direct
amplification disk, which was loaded onto the LIAISON® MDX instrument (DiaSorin).

20 Anti-Human Leucocyte-Antigen (HLA) Antibodies: Quantification and Analysis

21 Anti-HLA antibodies were identified with Luminex single antigen beads (One Lambda,

22 Canoga Park, CA) before UC-MSC infusion and on D14 in the HLA Immunology

23 Laboratory, Hôpital Saint-Louis, Paris, France (cut-off mean fluorescence intensity (MFI) >

24 500) on D0 and D14. Sera were tested using LabScreen Single Antigen Beads (SAB; One

25 Lambda). LabScreen assays were performed according to the manufacturer's protocol. After

1	sampling, blood was centrifuged (within 4 days), and immediately frozen at -30°C. Before
2	SAB testing, a 0.1-M solution of disodium EDTA (Sigma-Aldrich, St Louis, MO), pH=7.4,
3	was diluted 1:10 in the patient's serum and incubated for 10 minutes to avoid a prozone
4	effect. A preliminary adsorption with microparticles treated with blocking solution was done
5	to reduce high background noise caused by nonspecific binding of materials in human serum
6	to the latex beads used in flow-cytometry antibody-detection assays. 20 μ L of test serum were
7	pre-added to 5 μ L each of LS1A04 or LS2A01 SA beads, incubated in the dark for 30
8	minutes at room temperature, and then washed with wash buffer. 100 μ L of goat anti-human
9	IgG secondary antibody conjugated with R-phycoerythrin (PE), diluted 1:100, were added to
10	the beads, incubated for 30 minutes in the dark at room temperature, then washed and read on
11	the LABScan 200 flow cytometer (One Lambda). Panel reactive antibodies were calculated
12	(screened) with the unacceptable (background) antigens (MFI > 1000) using the
13	Eurotransplant Reference Laboratory (ETRL) virtual PRA calculator
14	(https://www.etrl.org/vPRA.aspx). A PRAc differing from 0% on D0, identified pre-formed
15	donor-specific antibodies (DSA) directed against the administered UC-MSCs in the absence
16	of immunising events (pregnancy, transfusion, organ transplantation). De novo DSA were
17	tracked on D14 based on increased anti-HLA immunisation with the presence of DSA
18	directed against the administered UC-MSCs.
19	

20 Statistical Analyses

Secondary analyses were considered exploratory. Time to death was compared between
groups using the log-rank test. In other time-to-event analyses, death was considered as a
competing event using the Fine & Gray approach.

24

25 Role of the Funding Source

1	The funder of the study had no role in the study design, data collection, data analysis, data
2	interpretation, or writing of this report. The corresponding author had full access to all the
3	study data and had final responsibility for the decision to submit for publication.
4	
5	
6	RESULTS
7	Characterization of UC-MSC-based Advanced Therapy Medicinal Product (ATMP)
8	Safety assays satisfied pre-set specifications for all batches, with the absence of
9	microbiological organisms, endotoxins (<2 EU/mL), and mycoplasma (<10 CFU/mL). Before
10	cell cryopreservation, each batch had a normal karyotype (Additional Table 1).
11	Characterization was consistent across batches (Additional Table 2 and 3).
12	
13	Plasma inflammatory cytokines, chemokines, growth Factors and biomarkers at
13 14	Plasma inflammatory cytokines, chemokines, growth Factors and biomarkers at baseline (D0) and D2, D4, D7, and D14 after starting infusions.
13 14 15	Plasma inflammatory cytokines, chemokines, growth Factors and biomarkers atbaseline (D0) and D2, D4, D7, and D14 after starting infusions.After Benjamini–Hochberg correction, biomarker concentrations did not differ between
13 14 15 16	Plasma inflammatory cytokines, chemokines, growth Factors and biomarkers atbaseline (D0) and D2, D4, D7, and D14 after starting infusions.After Benjamini–Hochberg correction, biomarker concentrations did not differ betweengroups at any time evaluated (Additional Figure 2A and 3). Over time, several cytokines and
13 14 15 16 17	Plasma inflammatory cytokines, chemokines, growth Factors and biomarkers atbaseline (D0) and D2, D4, D7, and D14 after starting infusions.After Benjamini–Hochberg correction, biomarker concentrations did not differ betweengroups at any time evaluated (Additional Figure 2A and 3). Over time, several cytokines andchemokines (RAGE, MCP-2, IP-10, IL-7, IL-10, IL-13, IL-18 TNF-α) in UC-MSC–group
13 14 15 16 17 18	Plasma inflammatory cytokines, chemokines, growth Factors and biomarkers atbaseline (D0) and D2, D4, D7, and D14 after starting infusions.After Benjamini–Hochberg correction, biomarker concentrations did not differ betweengroups at any time evaluated (Additional Figure 2A and 3). Over time, several cytokines andchemokines (RAGE, MCP-2, IP-10, IL-7, IL-10, IL-13, IL-18 TNF-α) in UC-MSC–grouppatients decreased significantly, but those diminutions appeared later (D7 and D14 vs D0)
13 14 15 16 17 18 19	Plasma inflammatory cytokines, chemokines, growth Factors and biomarkers atbaseline (D0) and D2, D4, D7, and D14 after starting infusions.After Benjamini–Hochberg correction, biomarker concentrations did not differ betweengroups at any time evaluated (Additional Figure 2A and 3). Over time, several cytokines andchemokines (RAGE, MCP-2, IP-10, IL-7, IL-10, IL-13, IL-18 TNF-α) in UC-MSC–grouppatients decreased significantly, but those diminutions appeared later (D7 and D14 vs D0)(Additional Figure 2B and 3) than the placebo group's pro-inflammatory cytokine and
13 14 15 16 17 18 19 20	Plasma inflammatory cytokines, chemokines, growth Factors and biomarkers atbaseline (D0) and D2, D4, D7, and D14 after starting infusions.After Benjamini–Hochberg correction, biomarker concentrations did not differ betweengroups at any time evaluated (Additional Figure 2A and 3). Over time, several cytokines andchemokines (RAGE, MCP-2, IP-10, IL-7, IL-10, IL-13, IL-18 TNF-α) in UC-MSC–grouppatients decreased significantly, but those diminutions appeared later (D7 and D14 vs D0)(Additional Figure 2B and 3) than the placebo group's pro-inflammatory cytokine andchemokine (RAGE, IP-10, MCP-3, MCP-1, MCP-2, CXCL9, M-CSF, IL-7, IL-5, IL-18, IL-
13 14 15 16 17 18 19 20 21	Plasma inflammatory cytokines, chemokines, growth Factors and biomarkers atbaseline (D0) and D2, D4, D7, and D14 after starting infusions.After Benjamini–Hochberg correction, biomarker concentrations did not differ betweengroups at any time evaluated (Additional Figure 2A and 3). Over time, several cytokines andchemokines (RAGE, MCP-2, IP-10, IL-7, IL-10, IL-13, IL-18 TNF-α) in UC-MSC-grouppatients decreased significantly, but those diminutions appeared later (D7 and D14 vs D0)(Additional Figure 2B and 3) than the placebo group's pro-inflammatory cytokine andchemokine (RAGE, IP-10, MCP-3, MCP-1, MCP-2, CXCL9, M-CSF, IL-7, IL-5, IL-18, IL-27, CTACK) concentrations, which declined significantly on D4 and D7 vs D0 (Additional
13 14 15 16 17 18 19 20 21 22	Plasma inflammatory cytokines, chemokines, growth Factors and biomarkers atbaseline (D0) and D2, D4, D7, and D14 after starting infusions.After Benjamini–Hochberg correction, biomarker concentrations did not differ betweengroups at any time evaluated (Additional Figure 2A and 3). Over time, several cytokines andchemokines (RAGE, MCP-2, IP-10, IL-7, IL-10, IL-13, IL-18 TNF-α) in UC-MSC–grouppatients decreased significantly, but those diminutions appeared later (D7 and D14 vs D0)(Additional Figure 2B and 3) than the placebo group's pro-inflammatory cytokine andchemokine (RAGE, IP-10, MCP-3, MCP-1, MCP-2, CXCL9, M-CSF, IL-7, IL-5, IL-18, IL-27, CTACK) concentrations, which declined significantly on D4 and D7 vs D0 (AdditionalFigure 2C and 3).

24 SARS-CoV-2 RT-PCR and SARS-CoV-2 N-Antigenemia Assay

25 Based on viral RNA levels, 13 (28.9%) patients had detectable viremia on D0 (Additional

1	Figure 4A), while 42 (93.3%) patients were N-antigenemia–positive (Additional Figure 4B).
2	The percentages of viremic patients and N-antigenemia levels decreased sharply until D4
3	(Additional Figure 4A–C). No between-group differences were observed for the percentages
4	of viremic patients and/or N-antigenemia diminutions over time up to D14.
5	
6	
7	References
8	1. Nicotra T, Desnos A, Halimi J, et al. Mesenchymal stem/stromal cell quality control:
9	validation of mixed lymphocyte reaction assay using flow cytometry according to ICH
10	Q2(R1). Stem Cell Res Ther 2020;11:426.
11	
12	Figure legend
13	Additional Figure 1. Survival probabilities and SOFA scores. (A) Survival rates from D0
14	to D28 were comparable for the two groups (P= 0.63 , log-rank test). (B) SOFA-score
15	evolutions from D0 to D28 did not differ (P= 0.79 , Wilcoxon test). Data are expressed as
16	mean per day±standard deviation. D day. SOFA Sequential Organ-Failure Assessment score.
17	UC-MSCs umbilical cord-derived mesenchymal stromal cells.
18	
19	Additional Figure 2. Analysis of plasma inflammatory cytokines, chemokines, growth
20	factors, and biomarkers at baseline (D0) and D2, D4, D7, and D14 after starting
21	infusions. Concentrations of 48 cytokines were quantified in plasma from UC-MSC– (n=20)
22	or placebo- treated (n =21) patients. Statistical analyses with Wilcoxon rank-sum tests
23	compared values between groups (A) and within each group (B and C) at each day indicated.
24	Volcano plots were generated for each comparison to show the log ₂ fold-changes relative to
25	placebo or D0, with statistical significance reported as -log ₁₀ P-values. Significance, defined

1	as $P < 0.05$, is in <i>blue</i> , with those remaining statistically significant after multiple corrections
2	(Benjamini–Hochberg correction) in red D day, D0 baseline, UC-MSC umbilical cord-derived
3	mesenchymal stromal cell, ANGP-1 angiopoietin-1, BCA-1 B-cell-attracting chemokine-1,
4	CTACK cutaneous T-cell-attracting chemokine, CXCL-9 chemokine (C-X-C motif) ligand 9,
5	EGF epidermal growth factor, FLT-3L Fms-related tyrosine kinase-3 ligand, G-CSF
6	granulocyte-colony–stimulating factor, $IFN-\gamma$ interferon- γ , IL interleukin, $IP-10$ interferon
7	gamma-induced protein-10, MCP monocyte chemoattractant protein, M-CSF macrophage-
8	colony-stimulating factor, MDC macrophage-derived chemokine, PDGF-AA platelet-derived
9	growth factor-AA, RAGE receptor for advanced glycation end products, sCD40L soluble
10	cluster of differentiation-40 ligand, SDF stromal cell-derived factor, SPD surfactant protein B,
11	TGF - α transforming growth factor- α , TPO thrombopoietin, $VEGF$ - A vascular endothelial
12	growth factor-A.

13

14 Additional Figure 3. Analysis of plasma inflammatory cytokine, chemokine, growth 15 factor, and biomarker concentrations on D0 (baseline), D2, D4, D7, and D14 after 16 starting infusions. The figure reports the quantification results for 10 cytokines selected among the 48 sought in plasma samples from patients treated with UC-MSCs (n=20) or 17 18 placebo (n=21). Data are log₂ transformed. Box plots of PaO₂/FiO₂ ratios: internal horizontal *lines* are the medians, *lower* and *upper box limits* are the 25th and 75th interquartile range, 19 respectively, vertical bars are drawn down to the 10th percentile and up to the 90th percentile. 20 D day, IL interleukin. IP-10 interferon-gamma-induced protein-10, MCP monocyte 21 22 chemoattractant protein, RAGE receptor for advanced glycation end products, SDF-1 stromal cell-derived factor- α , UC-MSCs umbilical cord-derived mesenchymal stromal cells. 23 24

1	Additional Figure 4. Analysis of plasma SARS–CoV-2 RNA and N-antigenemia levels at
2	baseline (D0) and D2, D4, D7, and D14 after starting infusions. Plasma SARS–CoV-2
3	RNA (by RT-PCR) and N-antigenemia in UC-MSC- (n=21, red) or placebo-treated (n=24,
4	blue) patients were quantified. Based on viral RNA levels (A), five (23.8%) UC-MSC- and
5	eight (33·3%) placebo-treated patients had detectable viremia on D0, while (B) N-
6	antigenemia was positive for 20 (95.2%) and 22 (91.7%) patients, respectively. (C) Plasma
7	SARS-CoV-2 NAg-level change from D0 to D14. Data are expressed as mean±standard
8	deviation. The percentage of viremic patients and N-antigenemia levels decreased sharply
9	until D4 (A–C). No between-group difference was observed in terms of percentage of viremic
10	patients and/or decline from D0 to D14. Red=UC-MSC group; blue=placebo group. D day,
11	PaO ₂ /FiO ₂ ratio of partial pressure of oxygen to fractional inspired oxygen, RT-PCR reverse
12	transcription-polymerase chain reaction, SARS-CoV-2 severe acute respiratory syndrome
13	coronavirus-2, UC-MSCs umbilical cord-derived mesenchymal stromal cells.

	Results	Specifications	Compliance rate
Identity			
UC-MSC×10 ⁶ /kg	0·9±0·1	1·0±0·1*	96%
Viability, %	78·4±5·3	≥70	100%
CD90, %	99·2±1·6	>90	100%
CD73, %	99·9±0·1	>90	100%
CD105, %	97·0±1·9	>90	100%
CD45, CD34, CD11b, CD19, HLA-DR, %	0·8±0·7	<2	100%
Safety			
Karyotype	Normal	Normal	100%
Microbiology	Negative	Negative	100%
Endotoxins	<2 EU/mL	<2 EU/mL	100%
Mycoplasma	<10 CFU/mL	<10 CFU/mL	100%

1 Additional Table 1: Quality-control characteristics of UC-MSCs used as treatment: identity and safety.

Values are expressed as mean±standard deviation. *1.00±0.1×106/kg with a maximum total dose of 80×106 UC-MSCs.

CD cluster of differentiation, EU endotoxin unit, CFU colony-forming unit, UC-MSCs umbilical cord-derived mesenchymal stromal cells.

1 Additional Table 2: Quality-control characteristics of batch-1 UC-MSCs used as treatment: identity and safety.

Criteria	Methods	Specifications	Results			
Viability	NC-200™	≥80%	95%	89%	86%	
Immunophenotype	Flow cytometry (BD Stemflow hMSC Analysis Kit)	Positive markers : CD90, CD73, CD105 \geq 90% Negative markers : CD45, CD34, CD11b, CD19, HLA-DR \leq 2%	CD90 : 99.96%, CD73 : 99.97% CD105 : 97.86% Negativ markers : 0.38%	CD90 : 99.67%, CD73 : 99.97% CD105 : 98.75% Negativ markers : 1.59%	CD90 : 99.85%, CD73 : 99.96% CD105 : 98.37% Negativ markers : 1.69%	
karyotype	G-banding	Normal over at least 20 mitosis	Conform	Conform	Conform	
Sterility	BactAlert	Negative	Negative	Negative	Negative	
Mycoplasma	qPCR (Kit Venor Gem qEP Minerva)	< 10 CFU/mL	< 10 CFU/mL	< 10 CFU/mL	< 10 CFU/mL	
Endotoxins	Kinetic colorimetry	< 2 EU/mL	< 2 EU/mL	< 2 EU/mL	< 2 EU/mL	
CFU-F	CFU-F assay	≥ 10/1000 nucleated cells	≥ 10/1000 nucleated cells	≥ 10/1000 nucleated cells	≥ 10/1000 nucleated cells	

2 3 4

CD cluster of differentiation, CFU colony-forming unit, CFU-F colony-forming unit–fibroblast, EU endotoxin unit, qPCR quantitative polymerase chain reaction, UC-MSCs umbilical cord-derived mesenchymal stromal cells.

5 Additional Table 3: Quality-control characteristics of batch-2 UC-MSCs used as treatment: identity and safety.

Criteria	Methods	Specifications		Results	
Viability	NC-200™	≥80%	88%	91%	92%
Immunophenotype	Flow cytometry (BD Stemflow hMSC Analysis Kit)	Positive markers : CD90, CD73, CD105 \geq 90% Negative markers : CD45, CD34, CD11b, CD19, HLA-DR \leq 2%	CD90: 99,77% CD73: 99,94% CD105: 99,24% CD45 / HLA-DR / CD19 / CD14 : 0,31%	CD90: 99,97% CD73: 99,89% CD105: 99,47% CD45 / HLA-DR / CD19 / CD14 : 1,28	CD90: 99,53% CD73: 99,86% CD105: 96,53% CD45 / HLA-DR / CD19 / CD14 : 1,23%
karyotype	G-banding	Normal over at least 20 mitosis	Conform	Conform	Conform
Sterility	BactAlert	Negative	Negative	Negative	Negative
Mycoplasma	qPCR (Kit Venor Gem qEP Minerva)	< 10 CFU/mL	< 10 CFU/mL	< 10 CFU/mL	< 10 CFU/mL
Endotoxins	Kinetic colorimetry	< 2 EU/mL	< 2 EU/mL	< 2 EU/mL	< 2 EU/mL
MLR	Evaluation of the lymphocyte proliferation inhibition by MSCs (flow cytometry) *	AUC*	0.1522	0.1381	0.1582
CFU-F	CFU-F assay	$\geq 10/1000$	$\geq 10/1000$	$\geq 10/1000$ nucleated cells	$\geq 10/1000$

6 7 8

*No published specifications.

AUC area under curve, CD cluster of differentiation, CFU colony-forming unit, CFU-F colony-forming unit–fibroblast, EU

endotoxin unit, qPCR quantitative polymerase chain reaction, UC-MSCs umbilical cord-derived mesenchymal stromal cells.

		UC-MSC (n=21)			Placebo (n=24)					
	D0	D3	D7	D14	D28	D0	D3	D7	D14	D28
Ventilatory support*	10 (47·6%)	9 (42·9%)	4 (19·0%)	1 (4·8%)	0 (0%)	4 (16·7%)	4 (16·7%)	2 (8·3%)	2 (8·3%)	0 (0%)
Invasive mechanical ventilation	11	12	12/20	7/16	4/4	20	19	16/22	10/16	5/6
	(52·4%)	(57·1%)	(60%)	(43·8%)	(100%)	(83·3%)	(79·2%)	(72·7%)	(62.5%)	(83·3%)
Tidal volume, mL/kg	6.2 (0.7)	7.0 (1.4)	7 (1·2)	6.0 (0.9)	5.7 (0.8)	6.3 (0.8)	6·8 (1·4)	7·1 (2·2)	7.0 (1.5)	6.7 (1.7)
Plateau airway pressure, cm H ₂ O	21.8 (4.2)	22.2 (5.4)	24.8 (7.0)	26.0 (5.7)	45·5 (2·1)	24.8 (5.0)	26.4 (8.3)	26.5 (2.4)	24.7 (8.6)	18 (NA)
PEEP	10.8 (2.9)	10.7 (3.7)	10.4 (3.9)	10.3 (4.6)	6·2 (1·9)	11·2 (3·2)	9.4 (3.2)	8.9 (3.7)	9.7 (4.2)	7.8 (4.2)
Driving pressure	11·3 (4·3)	11.4 (4.0)	15·2 (6·3)	16 (5·7)	38.5 (4.9)	13·2 (3·9)	16·2 (8·9)	15·5 (2·6)	14.0 (6.1)	13·0 (NA)
Compliance, mL/cm H ₂ O	45·2 (27·8)	42.9 (12.8)	37.6 (19.8)	26.6 (9.5)	11.1 (2.3)	35·2 (14·9)	34·6 (12·2)	27.7 (4.9)	44.8 (40.0)	52·1 (NA)
Neuromuscular blocking agents	6	9	6/19	3/14	1/4	16	14	7/20	5/15	1/6
	(28.6%)	(42.9%)	(31.6%)	(21·4%)	(25%)	(66.7%)	(58·3%)	(35%)	(33·3%)	(16·7%)
Ventilation mode										
Volume control	11/11	9/12	8/12	5/7	2/4	19/20	18/19	13/16	8/10	2/5
	(100%)	(75%)	(66.7%)	(71·4%)	(50%)	(95%)	(94.7%)	(81·3)	(80%)	(40%)
Pressure control	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Pressure support	0 (0%)	3 (25%)	4 (33·3)	2 (28.6%)	2 (50%)	1 (5%)	1 (5·2%)	3 (18·7%)	2 (20%)	3 (60%)

Additional Table 4: Evolution of respiratory characteristics from baseline (D0) to D28

Values are expressed mean (standard or number) (%). Denominators are indicated when values were missing. *D* day, *PaO*₂/*FiO*₂ ratio of partial pressure of oxygen to fractional inspired oxygen, *PEEP* positive end-expiratory pressure, *UC-MSCs* umbilical cord-derived mesenchymal stromal cells, *NA* not applicable. *Non-invasive ventilation and/or high-flow nasal oxygen therapy

Patient	Weight (kg)	Dose ×10 ⁶ UC-MSCs/kg (D1, D3±1, and D5±1)
R0003	100.0*	0.8 D1, D3, and D4
R0004	89.0*	0 [.] 9 D1, D3, and D4
R0008	75.0	1.0 D1, D4, and D6
R0123	91·0*	0.9 D1, D3, and D5
R0181	80.0	1.0 D1, and D3
S2009	94.0*	0 [.] 9 D1, D2, and D4
S2114	85·0*	0.9 D1
S2115	85·0*	0.9 D1, D4, and D6
S2118	92.0*	0.9 D1, and D4
S2119	70.0	1.0 D1, D3, and D4
S2121	85·0*	0.9 D1, D2, and D6
S2124	127.0*	0.6 D1, D3, and D4
S2125	87.0*	0.9 D1, D3, and D6
S2128	80.0	1.0 D1, D4, and D6
S2129	92.0*	0·7 D1 [†] , 0·8 D3, 0·9 D4
S2132	81·0*	1.0 D1, 1.0 D3, 1.0 D4
S2134	75.0	1.0 D1, 1.0 D3, 0.9 D4 [†]
S2189	87·0*	0.9 D1, D3, and D4
S2192	80.0	1.0 D1
S2194	85·0*	0.9 D1, D3, and D4
S2196	75.0	1·0 D1, 1·0 D3, 1·0 D6

Additional Table 5. UC-MSCs posology per patient

*Patients for whom 1·0±0·1×10⁶/kg reached a maximum total dose of 80×10⁶ MSC.
 [†]Doses below the pre-specified therapeutic dose (1·0±0·1×10⁶/kg, with a maximum total dose of 80×10⁶ MSC). *D* day, *UC-MSCs* umbilical cord-derived mesenchymal stromal cells.

Additional Table 6. Subgroup analyses of the primary outcome.

Imputation*	Sub group	UC-MSC (n = 21)	placebo (n = 24)	Estimate	р
1	MV = 0	58.7 [33.4;105.3]	95.2 [61.4;125.4]	0.4 [-133.6;134.4]	0.99
2	MV = 0	58.7 [33.4;101.9]	83.7 [61.4;101.7]	5.1 [-113.8;123.9]	0.93
1	MV = 1	54.3 [-45.7;78.3]	6.9 [-41.8;79.7]	0.6 [-64.6;65.7]	0.99
2	MV = 1	54.3 [-36.5;78.3]	6.9 [-41.8;69.8]	10.8 [-46.8;68.4]	0.70
1	PaO ₂ /FiO ₂ ratio > 150	-12.7 [-76.2;70.8]	71.7 [-52.6;110]	-4.6 [-105.6;96.4]	0.93
2	PaO ₂ /FiO ₂ ratio > 150	-12.7 [-62.1;70.8]	69.2 [-52.6;97.6]	-4.8 [-94.8;85.1]	0.91
1	PaO_2/FiO_2 ratio ≤ 150	63.0 [54.3;100.6]	17.9 [-7.2;53.7]	39.2 [-8.4;86.8]	0.10
2	PaO_2/FiO_2 ratio ≤ 150	63.0 [54.3;100.6]	17.9 [-7.2;53.7]	41.5 [-1.2;84.3]	0.06
1	Age < 65 years	73.8 [47.9;109.9]	77.1 [-2.5;112.3]	27.9 [-63.9;119.8]	0.53
2	Age < 65 years	73.8 [47.9;109.9]	70.4 [-2.5;85.8]	31.3 [-49.4;112.1]	0.42
1	Age ≥ 65 years	46.7 [-51.8;63.3]	-18.6 [-60.8;37.9]	10.6 [-59.6;80.9]	0.76
2	Age ≥ 65 years	46.7 [-51.8;63.3]	-18.6 [-60.8;37.9]	20.0 [-42.0;82.0]	0.51
1	DSA negative among treated group	58.8 [-42.7;107.3]	25.3 [-33.3;104.6]	13.9 [-48.3;76.1]	0.65
2	DSA negative among treated group	58.8 [-42.7;107.3]	25.3 [-33.3 ;83.1]	18.4 [-36.1;72.9]	0.50

Values are median [interquartile range]. *MV* mechanical ventilation, *DSA* donor-specific antigen, *UC-MSCs* umbilical cord-derived mesenchymal stromal cells.

**Imputation 1*: PaO2/FiO2 ratios of patients who died were imputed to 50, whereas those who improved and left the ICU before D7 were imputed using their last observed value plus 10%.

*Imputation 2: A sensitivity analysis was computed using a last observed carried forward approach.

Additional Table 7. Complete list of adverse events.

Adverse events	UC-MSCs	Placebo	Total
Edema	0 (0%)	1 (1.4%)	1 (0.7%)
Atrial Fibrillation	1 (1.5%)	0 (0%)	1 (0.7%)
Metabolic acidosis	1 (1.5%)	0 (0%)	1 (0.7%)
Worsening hypercapnia	0 (0%)	1 (1.4%)	1 (0.7%)
Worsening hepatic cytolysis	0 (0%)	1 (1.4%)	1 (0.7%)
Worsening shock	0 (0%)	1 (1.4%)	1 (0.7%)
Worsening interstitial syndrome	0 (0%)	1 (1.4%)	1 (0.7%)
Delirium	2 (2.9%)	1 (1.4%)	3 (2.1%)
Metabolic alkalosis	1 (1.5%)	0 (0%)	1 (0.7%)
Anemia	1 (1.5%)	0 (0%)	1 (0.7%)
Anemia requiring transfusion	0 (0%)	1 (1.4%)	1 (0.7%)
Anemia – Hb 7.8 g/dL	1 (1.5%)	0 (0%)	1 (0.7%)
Microscopic hematuria	1 (1.5%)	0 (0%)	1 (0.7%)
Cardiac arrest and left pneumothorax	1 (1.5%)	0 (0%)	1 (0.7%)
Increase in triglycerid and lactatate-deshydrogenase levels	0 (0%)	1 (1.4%)	1 (0.7%)
Pseudomonas aeruginosa bacteremia	1 (1.5%)	0 (0%)	1 (0.7%)
Bronchospasm	0 (0%)	1 (1.4%)	1 (0.7%)
Hypovolemic shock	1 (1 5%)	0 (0%)	1 (0 7%)
Refractory sentic shock with bacteremia	0 (0%)	1 (1 4%)	1 (0 7%)
Sentic shock related to baemonbilus pneumonia	0 (0%)	1 (1.4%)	1 (0 7%)
Refractory sentic shock associated with multiple organ failure	1 (1 5%)	0 (0%)	1 (0 7%)
Cholestasis	1 (1.5%)	0 (0%)	1 (0.7%)
Fall from standing position	0 (0%)	1 (1 4%)	1 (0.7%)
	1 (1 5%)	0 (0%)	1 (0.7%)
	1 (1.5%)	0 (0%)	1 (0.7%)
Terminal extubation for irreversible encentral anathy	1 (1.5%)	0 (0%)	1 (0.7%)
Psychiatric decompensation	1 (1.5%)		1 (0.7%)
Acute respiratory distress requiring intubation and mechanical ventilation	1 (1.5%)	0 (0%)	1 (0.7%)
Shock	1 (1.5%)		F (3.6%)
Multiple organ failure	1 (1.5%)	-4(3.0%)	1 (0 7%)
Pofractory multiple organ failure	0 (0%)		1 (0.7%)
		1 (1.47)	1 (0.7%)
		0 (0%)	1 (0.7%)
			1 (0.7%)
Worsening shock		1 (1.470)	1 (0.7%)
Severe wereening shock and reeniretery distress		1 (1.470)	1 (0.7%)
	1 (1.5%)	0 (0%)	1 (0.7%)
Worsening respiratory status with level	1 (1.5%)		1 (0.7%)
Worsening respiratory distress requiring intubation and mechanical ventilation			1 (0.7%)
vorsening nemodynamic status		1 (1.4%)	1 (0.7%)
Delirium and neuromyopathy	1 (1.5%)		1 (0.7%)
		1 (1.4%)	1 (0.7%)
Respiratory exhaustion - hypoxemia	1 (1.5%)		1 (0.7%)
Dialysis	0 (0%)	1 (1.4%)	1 (0.7%)
Diarnnea		1 (1.4%)	1 (0.7%)
Liquid diarrhea	1 (1.5%)	0 (0%)	1 (0.7%)
Increase in uremia	0 (0%)	1 (1.4%)	1 (0.7%)
Acute pulmonary empolism		1 (1.4%)	1 (0.7%)
Pseudomonas aeruginosa- related ventilator-associated pneumonia	1 (1.5%)	0 (0%)	1 (0.7%)
Ihrombopenia	0 (0%)	1 (1.4%)	1 (0.7%)
Isolated thrombopenia	0 (0%)	1 (1.4%)	1 (0.7%)
Right occipital eschar	0 (0%)	1 (1.4%)	1 (0.7%)
Labbial eschar	1 (1.5%)	0 (0%)	1 (0.7%)
Heel, seat, elbow pressure sores	1 (1.5%)	0 (0%)	1 (0.7%)

Adverse events	UC-MSCs	Placebo	Total
Occipital eschar stade 4	0 (0%)	1 (1.4%)	1 (0.7%)
Fever	0 (0%)	1 (1.4%)	1 (0.7%)
Fever 39.1°C	1 (1.5%)	0 (0%)	1 (0.7%)
Fever 39.5°C	1 (1.5%)	0 (0%)	1 (0.7%)
Staphylococcus epidermidis bacteremia	0 (0%)	1 (1.4%)	1 (0.7%)
Staphylococcus haemolyticus bacteremia	0 (0%)	1 (1.4%)	1 (0.7%)
Streptococcus mitis bacteremia	1 (1.5%)	0 (0%)	1 (0.7%)
Hyperkalemia without renal failure	1 (1.5%)	0 (0%)	1 (0.7%)
Persistent hyperkalemia related to acidosis	0 (0%)	1 (1.4%)	1 (0.7%)
Hyperleukocytosis	0 (0%)	1 (1.4%)	1 (0.7%)
Hyperlipasemia	1 (1.5%)	0 (0%)	1 (0.7%)
Arterial hypertension	0 (0%)	1 (1.4%)	1 (0.7%)
Hypertension	1 (1.5%)	0 (0%)	1 (0.7%)
Hypoalbumonemia	1 (1.5%)	0 (0%)	1 (0.7%)
Hypotension	1 (1.5%)	0 (0%)	1 (0 7%)
Severe edema	0 (0%)	1 (1 4%)	1 (0.7%)
Infection related to Pseudomonas aeruginosa and Klehsiella	0 (0%)	1 (1.4%)	1 (0.7%)
	1 (1 5%)	0 (0%)	1 (0.7%)
Sentic shock with respiratory failure	1 (1.5%)	0 (0%)	1 (0.7%)
	1 (1.5%)	1 (1 /%)	2 (1.4%)
	1 (1.576) 0 (0%)	1 (1.470)	2 (1.476)
	0 (0%)	1 (1.470)	1 (0.7%)
Functional cauta kidnov injuny	1 (1 5%)	0 (0%)	1 (0.7%)
Stable soute ronal failure			1 (0.7%)
Stable acute renarialiture		1 (1.4%)	1 (0.7%)
Wild Hypothatrennia	I (1.5%)	0(0%)	1 (0.7%)
	0 (0%)	1 (1.4%)	1 (0.7%)
Lymphanglus Dicht ann han ritir	0 (0%)	1 (1.4%)	1 (0.7%)
	0 (0%)	1 (1.4%)	1 (0.7%)
Nausea and diarrnea	1 (1.5%)		1 (0.7%)
Severe neuromyopatny		1 (1.4%)	1 (0.7%)
very severe neuromyopatny	1 (1.5%)		1 (0.7%)
Intensive care unit-associate neuromyopathy		1 (1.4%)	1 (0.7%)
Moderate neuromyopathy	1 (1.5%)		1 (0.7%)
New onset of Pseudomonas aeruginosa-related VAP	0 (0%)	1 (1.4%)	1 (0.7%)
Post-extubation laryngeal edema	0 (0%)	1 (1.4%)	1 (0.7%)
Alveolo-interstitiel syndrome	0 (0%)	1 (1.4%)	1 (0.7%)
Pansinusitis requiring surgery	1 (1.5%)	0 (0%)	1 (0.7%)
Right Pseudomonas aeruginosa-related VAP	0 (0%)	1 (1.4%)	1 (0.7%)
Pseudomonas aeruginosa- and Klebsiella-related VAP	1 (1.5%)	0 (0%)	1 (0.7%)
Staphylococcus aureus- and Haemophilus-related VAP	1 (1.5%)	0 (0%)	1 (0.7%)
Staphylococcus aureus-related VAP	0 (0%)	1 (1.4%)	1 (0.7%)
Citrobacter- and Serratia-related VAP	0 (0%)	1 (1.4%)	1 (0.7%)
Methicillin-sensitive Staphylococcus aureus-related VAP	0 (0%)	1 (1.4%)	1 (0.7%)
Late Klebsiella-related ventilator-associated pneumonia	0 (0%)	1 (1.4%)	1 (0.7%)
Febrile peak	0 (0%)	1 (1.4%)	1 (0.7%)
Fever 38.2°C	0 (0%)	1 (1.4%)	1 (0.7%)
Hypertensive peak	1 (1.5%)	0 (0%)	1 (0.7%)
VAP related to oropharyngeal flora	0 (0%)	1 (1.4%)	1 (0.7%)
VAP	1 (1.5%)	0 (0%)	1 (0.7%)
Late ventilator-associated pneumonia related to Citrobacter	1 (1.5%)	0 (0%)	1 (0.7%)
VAP related to oropharyngeal flora	0 (0%)	1 (1.4%)	1 (0.7%)
Healthcare-associated pneumonia	0 (0%)	1 (1.4%)	1 (0.7%)
Pneumonia related to oropharyngeal flora	0 (0%)	1 (1.4%)	1 (0.7%)
VAP related to multidrug-resistant bacteria	1 (1.5%)	0 (0%)	1 (0.7%)

Adverse events	UC-MSCs	Placebo	Total
VAP related to oropharyngeal flora	0 (0%)	1 (1.4%)	1 (0.7%)
VAP related to Hafnia and Proteus	0 (0%)	1 (1.4%)	1 (0.7%)
VAP related to Klebsiella	1 (1.5%)	0 (0%)	1 (0.7%)
VAP related to Pseudomonas spp.	1 (1.5%)	0 (0%)	1 (0.7%)
Extracorporeal membrane oxygenator implantation	1 (1.5%)	0 (0%)	1 (0.7%)
Refractory acute respiratory distress syndrome	0 (0%)	1 (1.4%)	1 (0.7%)
Bacteremia related to cocci Gram+ bacteria	1 (1.5%)	0 (0%)	1 (0.7%)
Refractory acute respiratory distress syndrome and multiple organ failure	0 (0%)	1 (1.4%)	1 (0.7%)
Superinfection related to Moraxella	1 (1.5%)	0 (0%)	1 (0.7%)
Suspected pulmonary embolism	0 (0%)	1 (1.4%)	1 (0.7%)
Suspected propofol-related infusion syndrome	0 (0%)	1 (1.4%)	1 (0.7%)
Suspected S.aureus-associated endocarditis	1 (1.5%)	0 (0%)	1 (0.7%)
Bilateral alveolo-interstitial syndrome	1 (1.5%)	0 (0%)	1 (0.7%)
Biological inflammatory syndrome	1 (1.5%)	0 (0%)	1 (0.7%)
Tachycardia	1 (1.5%)	2 (2.8%)	3 (2.1%)
Effort-related tachycardia	2 (2.9%)	0 (0%)	2 (1.4%)
Increase in D-Dimer	1 (1.5%)	0 (0%)	1 (0.7%)
High level of D-Dimer	1 (1.5%)	0 (0%)	1 (0.7%)
Thrombocytemia	0 (0%)	1 (1.4%)	1 (0.7%)
Thrombopenia	0 (0%)	1 (1.4%)	1 (0.7%)
Tracheostomy for mechanical ventilation weaning	1 (1.5%)	0 (0%)	1 (0.7%)
Vomitting	1 (1.5%)	0 (0%)	1 (0.7%)
Total	68 (48.9%)	71 (51.1%)	139 (100%)

Values are expressed as number (%). Hb hemoglobin, VAP ventilator-associated pneumonia.

	UC-MSC (n=21)	Placebo (n=24)	Total
Pre-specified AEs occurring during treatment infusion			
A clinical scenario consistent with transfusion incompatibility or	0 (0%)	0 (0%)	0 (0%)
transfusion-related infection			
Reaction necessitating treatment stoppage	0 (0%)	0 (0%)	0 (0%)
Pre-specified AEs occurring within 6 h of treatment onset			
Vasopressor-dose Increase	0 (0%)	1 (4.2%)	1 (2.2%)
New ventricular tachycardia or fibrillation, or asystole	0 (0%)	1 (4.2%)	1 (2.2%)
Worsening hypoxemia	0 (0%)	0 (0%)	0 (0%)
Pre-specified AE occurring within 24 h of treatment infusion			
Any cardiac arrest or death	0 (0%)	0 (0%)	0 (0%)

Additional Table 8. Summary of pre-specified infusion-associated adverse events for randomized subjects

Values are expressed as number (%). AE adverse event, UC-MSCs umbilical cord-derived mesenchymal stromal cells.

ADDITIONAL FIGURE 1

А



В

UC-MSCs placebo







ADDITIONAL FIGURE 3

ADDITIONAL FIGURE 4



III. Conclusion

1. Résumé des principaux résultats

L'essai clinique thérapeutique STROMA-CoV-2 est l'une des rares études randomisées, réalisée chez les patients infectés par le SARS-CoV-2 et ayant un SDRA tel que défini par les critères de Berlin, avec une méthodologie en double aveugle et surtout un timing homogène par rapport au début du SDRA. Cette étude permet de confirmer la sécurité clinique de l'utilisation de CSM-CO pour le traitement du SDRA lié au COVID-19. Bien qu'ayant des propriétés immunomodulatrices, cette thérapie cellulaire n'a pas retardé la clairance du virus (antigénémie ou virémie). Cet essai clinique montre aussi une absence d'effet indésirable grave notamment l'absence de survenue de thrombose pulmonaire symptomatique compte tenu de l'immunopathologie du SDRA lié au COVID-19 associée à une coagulopathie de petits vaisseaux (16,122,123). De plus, les CSM-CO produites au centre MEARY ont montré une faible immunogénicité dans ce contexte malgré une expression significative d'antigènes HLA-I.

La capacité de production de cette thérapie cellulaire en quantité suffisante dans le contexte de l'urgence a été un des principaux facteurs de la limitation de la taille de la population de l'étude et donc de la puissance de cet essai clinique innovant. Cependant, notre étude suggère une discrète amélioration du rapport PaO₂/FiO₂ sept jours après le traitement par CSM-CO qui nécessiterait une confirmation dans une étude de phase III. L'essai STROMA-CoV-2 a également permis de souligner le défi que représente la capacité de produire à grande échelle et sur une période de temps réduite ce médicament de thérapie innovante de grade clinique.

2. Perspectives

Au-delà de la faible puissance de notre étude, plusieurs questions sur la biologie des CSM-CO nécessitent d'être résolues pour optimiser le développement clinique de cette thérapeutique. La limitation de la posologie maximale (ici à 80x10⁶ CSM/perfusion), du fait d'une crainte d'obstruction vasculaire pulmonaire liée au « *piégeage* » pulmonaire préférentiel après injection (111,112), pourrait aussi expliquer l'absence d'efficacité de ce traitement dans notre étude par rapport à celle Lanzoni et al (121). Il sera intéressant d'évaluer la sécurité thromboembolique de ce traitement en cas d'utilisation de doses adaptées au poids mais non plafonnées. Plusieurs stratégies seront également à explorer pour augmenter la balance entre le principe actif apporté et ce risque vasculaire: 1) l'utilisation d'un substitut des CSMs cultivés

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in vitro (dont le diamètre est de l'ordre de 15 à 30 μ m) que sont les vésicules extracellulaires dérivées de CSMs (dont le diamètre est de l'ordre du 30 nm à 1 μ m) (124,125); 2) l'utilisation d'une voie d'administration nébulisée afin de limiter le passage intravasculaire (donc la limitation du risque thrombotique et du contact avec les anticorps anti-HLA préformés qui favorisent la clairance des CSMs), mais surtout de concentrer le principe actif des CSMs dans le tissu pulmonaire lésé.

Il est clair que les capacités immunomodulatrices et réparatrices des CSMs observées in vitro plaident pour un développement de cette thérapeutique en clinique notamment dans le SDRA lié au COVID-19. Néanmoins, une question actuellement non résolue reste l'identification de biomarqueurs in vitro prédictifs du comportement in vivo des CSMs. La variabilité fonctionnelle de cette thérapie est cependant dépendante du donneur, du nombre d'expansions cellulaires in vitro, et probablement du receveur (126–129). L'utilisation de CSMs issues d'iCSPs est une stratégie d'avenir du fait d'un certain nombre de bénéfices. Une seule banque d'iCSP permet de produire environ 29 millions de doses (chaque dose ayant 10⁸ CSMs) donc pour un seul donneur la possibilité d'avoir un nombre suffisant de CSMs pour traiter l'ensemble des patients d'un essai clinique à grande échelle (y compris dans le contexte de l'urgence thérapeutique) (130). Par ailleurs, lors de la production de CSMs à partir d'iCSPs les expansions cellulaires sont faites en amont du stade de différenciation en CSMs ce qui limite, contrairement aux protocoles actuels de production de CSMs à partir du cordon ombilical, la variabilité fonctionnelle épigénétique entre les lots que peuvent induire les expansions répétées des CSMs (130,131). Les travaux récents ont montré que l'analyse multiparamétrique in vitro du sécrétome et du transcriptome pourrait permettre de prédire et de choisir les lots cellulaires associés à une réponse clinique dans le cadre de la sclérodermie systémique (132). Il sera également crucial d'évaluer l'impact du microenvironnement in vivo au cours du SDRA lié au COVID-19 sur la fonctionnalité in vivo des CSMs. L'évaluation in vitro du potentiel immunomodulateur des cellules utilisées pour notre essai clinique a montré une potentialisation franche de la sécrétion de molécules immunomodulatrices telles que l' IDO (Indoleamine 2,3-dioxygenase) en réponse à un environnement inflammatoire tel que la présence in vitro d'IFN-y (133). En effet, il n'est pas exclu que les capacités fonctionnelles observées in vitro et bénéfiques pour le SDRA soit inhibées directement ou indirectement par le SARS-CoV-2 ou certaines thérapeutiques du SDRA lié au COVID-19 comme la corticothérapie (134). Le développement de simulation de la condition «in vivo » via la technologie des

organoïdes ou du poumon perfusé ex vivo sera une piste d'avenir car elle pourrait tenir compte de la majorité du microenvironnement cellulaire mais aussi humoral parfois non configurable in vitro (135–137). Enfin, l'évaluation des capacités régénératives et antifibrosantes des CSMs, actuellement en cours dans le cadre de l'étude STROMA-CoV-2, nous renseignera sur l'impact du traitement par CSM-CO sur la survenue de la fibrose pulmonaire (scanner thoracique) et la capacité fonctionnelle respiratoire (explorations fonctionnelles respiratoires à moyen et long terme). Les travaux de Shi et al ont suggéré un bénéfice dans l'amélioration des lésions pulmonaires à 28 jours et à 1 an après le traitement chez des patients hospitalisés avec une insuffisance respiratoire aiguë, mais non intubés, sans état de choc et sans défaillance d'organe dans le cadre de l'infection au SARS-CoV-2 (118,119). La question du timing d'introduction du traitement au cours du SDRA reste un enjeu complexe car il se pourrait que la fenêtre d'introduction de ce traitement soit trop tardive dans notre étude actuelle. Une analyse multiomique (génomique, transcriptomique, métabolomique, microbiomique) pourrait identifier lors de prochains essais cliniques, les marqueurs clinicoradio-biologiques prédictifs de réponse au traitement par CSMs. Cette approche aurait pour objectif le développement de test « point of care » pour sélectionner les patients avec un diagnostic de SDRA éligible à la thérapie par CSMs.

Au total, la thérapie par CSMs n'est pas encore prête pour une utilisation courante clinique dans le SDRA. Un certain nombre d'éléments comme la dose, le timing d'introduction du traitement, les facteurs prédictifs d'efficacité in vivo pour la sélection des lots cellulaires et de patients, l'impact du virus sur les propriétés thérapeutiques des CSMs et le profil fonctionnel in vivo des CSMs (analyse des exosomes) restent à explorer et à optimiser pour mieux positionner ce médicament dans le SDRA lié au COVID-19. L'étape suivante pourrait donc consister en la réalisation d'une phase III, ou d'un essai clinique randomisé triple aveugle multicentrique contrôlé contre placebo avec bras parallèles chez des patients COVID-19 avec un SDRA (critères de Berlin) de moins de 24h. Les patients pourraient recevoir 3 perfusions sur 3 jours consécutifs de 2x10⁶ CSM/kg/perfusion de « *iCSMs* » (CSM dérivés d'iCSP). Les patients pourraient être évalués sur un critère de jugement principal composite associant la mortalité à 28 jours après le traitement, l'incidence du recours à l'intubation au cours de l'hospitalisation, le nombre de jours sans ventilation mécanique. Une analyse multiomique (cellulaire et humorale) du LBA et du sang à 48h sera capitale car les travaux récents de Wick et al montrent qu'il existe une discordance entre la compartiment sanguin et alvéolaire sur les

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biomarqueurs de SDRA (101). L'analyse omique conjointe des « *iCSMs* » et des cellules des patients pourrait permettre l'identification de facteurs de sélection de lot cellulaire et de patients à fort risque de réponse à ce traitement.

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V. Résumé de la thèse

Contexte : Le syndrome de détresse respiratoire aiguë (SDRA) induit par le virus SARS–CoV-2 (*Severe Acute Respiratory Syndrome Coronavirus-2*) garde une mortalité importante. Les cellules stromales mésenchymateuses dérivées du cordon ombilical (CSM-CO) peuvent exercer des propriétés immunomodulatrices mais leur bénéfice dans le traitement du SDRA n'est pas statué. Cet essai clinique de phase 2b a donc été conduit pour évaluer cette thérapie cellulaire dans la prise en charge des patients ayant un SDRA lié au SARS–CoV-2.

Méthodes : cet essai multicentrique, en double aveugle, randomisé contrôlé contre placebo (STROMA–CoV-2, *www.clinicaltrials.gov* (*NCT04333368*)) a inclus des patients adultes (\geq 18 ans) ayant un SDRA lié au SARS–CoV-2 en phase précoce (<96 heures) dans 10 hôpitaux français. Les patients ont été randomisés pour recevoir, soit des perfusions intraveineuses de 3×10⁶ CSM-CO/kg soit le placebo (0,9% NaCl) pendant 5 jours. Une analyse en intention de traiter modifiée a été réalisée avec comme critère de jugement principal la variation du ratio de la pression partielle artérielle de dioxygène par la fraction inspirée de dioxygène (PaO₂/FiO₂), entre le début (J0) et le 7ème jour (J7) de l'étude.

Résultats : Parmi les 107 patients criblés pour éligibilité du 6 avril 2020 au 29 octobre 2020, 45 ont été inclus, randomisés et analysés. Les variations de PaO_2/FiO_2 entre J0 et J7 ne différaient pas significativement entre les groupes CSM-CO et placebo (médianes [écart interquartile] 54·3 [–15·5 à 93·3] vs 25·3 [–33·3 à 104· 6], respectivement ; ANCOVA 7·4, IC à 95 % –44·7 à 59·7 ; p=0·77). Six (28·6%) des 21 patients recevant le traitement par CSM-CO et six des 24 (25%) patients du groupe placebo ont eu des événements indésirables graves.

Conclusion : Les changements de PaO₂/FiO₂ de JO à J7 des patients avec SDRA lié au SARS– CoV-2 et traités par CSM-CO ne différaient pas de manière significative des patients du groupe placebo. Des essais plus importants incluant des patients plus tôt au cours de leur SDRA sont nécessaires pour évaluer davantage l'efficacité de la thérapie cellulaire par CSM-CO.

Mots clés : severe acute respiratory syndrome coronavirus-2, syndrome de détresse respiratoire aigüe, cellule stromale mésenchymateuse, cordon ombilical, immunomodulation

Serment d'Hippocrate

▓⇔▓⇔▓

En présence des Maîtres de cette école, de mes chers condisciples et devant l'effigie d'Hippocrate, je promets et je jure d'être fidèle aux lois de l'honneur et de la probité dans l'exercice de la médecine. Je donnerai mes soins gratuits à l'indigent et n'exigerai jamais un salaire au-dessus de mon travail. Admis dans l'intérieur des maisons mes yeux ne verront pas ce qui s'y passe ; ma langue taira les secrets qui me seront confiés, et mon état ne servira pas à corrompre les mœurs ni à favoriser le crime. Respectueux et reconnaissant envers mes Maîtres, je rendrai à leurs enfants l'instruction que j'ai reçue de leurs pères.

Que les hommes m'accordent leur estime si je suis fidèle à mes promesses ! Que je sois couvert d'opprobre et méprisé de mes confrères si j'y manque !

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