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(71) Applicant: **AMNIOTICS AB** [SE/SE]; Scheelevägen 2, 223 63 Lund (SE).

(72) Inventors: **LARSSON, Marcus Kare Torleif**; Scheelevägen 2, SE-223 63 Lund (SE). **TALTS, Jan**; Scheelevägen 2, SE-223 63 Lund (SE).

(74) Agent: **STRÖM & GULLIKSSON AB**; P.O. Box 4188, S-203 13 Malmö (SE).

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(57) Abstract: The present invention relates to mesenchymal stem cells derived from term amniotic fluid that have been selected for one or more neural markers (in particular, the surface marker OSCAR (osteoclast associated Ig-like receptor)), and their uses in the nervous system.

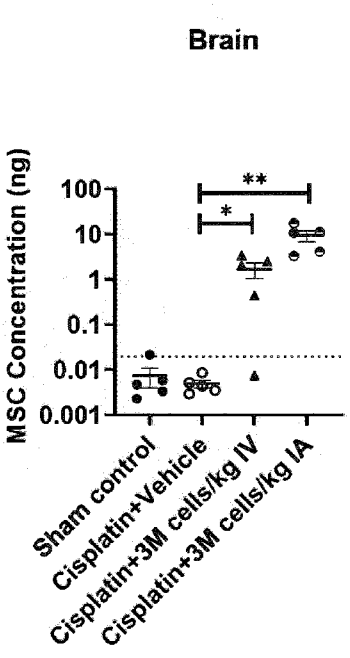

Figure 7

WO 2024/218331 A1

Mesenchymal stem cells and uses thereof

FIELD OF THE INVENTION

The present invention relates to mesenchymal stem cells derived from term amniotic fluid that have been selected for one or more neural markers, and their uses in the nervous system.

BACKGROUND

Mesenchymal stem cells (MSCs) can be found in nearly all tissues and are mostly located in perivascular niches. As will be understood by one of skill in the art, MSCs are multipotent stromal cells capable of differentiating into numerous cell types, and possess anti-inflammatory, angiogenic properties for directing tissue repair processes, thereby making MSCs valuable for therapeutic treatments. Term amniotic fluid (TAF) collected during a caesarean section contains several valuable cells, including MSCs. Moreover, specific subpopulations of MSCs are likely to be particularly well suited to use for production of therapeutic drugs. Previously, MSCs sourced from adult bone marrow, adult adipose tissue or neonatal birth-associated tissues including placenta, umbilical cord and cord blood were extensively used to obtain MSCs. MSCs from these neonatal tissues may have additional capacities in comparison to MSCs derived from adult sources. Indeed, several studies have reported superior biological properties such as improved proliferative capacity, life span and differentiation potential of MSCs from birth-associated tissues over adult derived MSCs.

MSCs are attractive therapeutic agents due to their potential to target multiple pathways involved in neuronal regeneration as well as an established safety profile. MSCs intrinsically possess unique features enabling them to migrate toward areas of inflammation. However, the blood-brain barrier may pose a barrier for entry to the brain.

Targeting drugs to the brain faces difficulties, in particular due to the blood-brain barrier (BBB), which restricts transport to many molecules. Approved drugs that are capable of crossing the BBB are generally limited to lipid-soluble small molecules. Alternative means to target the brain include invasive delivery methods such as injection into the cerebrospinal fluid (CSF) following intrathecal or trans-nasal administration, or by trans-cranial directed injection of a drug into the brain tissue by intracerebral administration (Pardridge, 2022); or by disrupting the BBB, for example by using noxious agents.

However, such invasive approaches can be discomforting to a patient, and use of noxious agents may incur risky side effects or issues due to induced disruption of the BBB.

As reviewed extensively in Pardridge, 2022, the challenges in delivering to the brain also applies to cell-mediated BBB transport. Although some literature proposes that MSCs can cross the BBB, these studies fail to support that MSCs can cross the intact BBB. For example, in one study of a spinal cord lesion model, the stem cells were injected directly into the spinal cord, which allows the cells to bypass the BBB (Akiyama *et al.*, 2002). There has been a failure to observe the homing or trafficking of MSCs into the brain following intravenous administration at sites where the BBB is present (Soper *et al.*, 2004). Although human-induced pluripotent stem cells (iPSCs) have been considered a potential solution to the issue of cells traversing the BBB, there is currently no evidence that iPSCs selectively cross the BBB relative to MSCs (Pardridge, 2022).

A number of neural pathologies may disrupt the BBB, making it leakier and more accessible to agents that would otherwise fail to pass the BBB. However, such damage is often indicative of a later point of neural disease progression, by which time the available therapies may be less effective.

Accordingly, there is a need in the art for improved means to target the brain. For example, by having a therapeutic able to cross the intact BBB, one may begin addressing pathology prior to damage occurring to the BBB.

SUMMARY OF THE INVENTION

Accordingly, it is an object of the present invention to advance the utility of MSCs for targeting the CNS, by providing improved MSC sources that are adapted for homing and/or trafficking to the CNS, for example by have the capability of passing an intact BBB.

A first aspect of the present invention provides an isolated population of mesenchymal stem cells (MSCs) for use in treating or preventing a disease, disorder or condition of the nervous system in a subject in need thereof. Preferably, the MSCs have been obtained from term amniotic fluid (TAF), which may be referred to herein as "TAF MSCs" or "TAF-MSCs". In some embodiments, the MSCs have been selected for the expression of at least one surface marker selected from the group consisting of OSCAR, HAVCR1, ACKR3, C3, SIRPB1, SLC6A6, CCKAR, TNFSF10, CLSTN2, TENM2, SFRP1, PIK3IP1, SCNN1D, CLDN11, ALDH3B1 and ITGB4. Preferably, the MSCs have been selected based on the expression

of OSCAR (which may be referred to herein as OSCAR+ MSCs, OSCAR+ TAF MSCs or OSCAR+ cells).

A second aspect of the present invention provides a pharmaceutical composition (also referred to herein as a "composition") comprising an isolated population of MSCs and a pharmaceutically acceptable excipient, diluent or carrier for use in treating or preventing a disease, disorder or condition of the nervous system in a subject in need thereof. The MSCs may be defined according to the first aspect of the present invention.

A third aspect of the present invention provides a kit comprising an isolated population of MSCs or a pharmaceutical composition comprising an isolated population of MSCs, wherein the kit further comprises instructions for use in treating or preventing a disease, disorder or condition of the nervous system. The MSCs may be defined according to the first aspect of the present invention, and/or in the form of the second aspect of the present invention. In some embodiments, the kit further comprises a reconstitution buffer and/or at least one drug or agent. The kit may further comprise instructions for use. In some embodiments, the components of the kit are housed in separate containers.

A fourth aspect of the present invention provides a method of treating or preventing a disease, disorder or condition of the nervous system in a subject in need thereof, wherein the method comprises administering MSCs, and wherein the MSCs are administered at a location such that the MSCs home to and/or cross the BBB to exert a therapeutic effect to treat or prevent the disease, disorder or condition of the nervous system. The MSCs may be defined according to the first aspect of the invention, or in the forms according to the second and/or third aspects of the present invention.

A fifth aspect of the present invention provides a method of making an isolated population of MSCs. The MSCs may be defined according to the first aspect of the invention.

In some embodiments, the disease, disorder or condition of the nervous system is of the central nervous system (CNS). In some embodiments, the disease, disorder or condition of the CNS is selected from the group consisting of: arachnoid cysts, brain tumours (including, for example, glioma or glioblastoma), catalepsy, encephalitis, epilepsy, neural infection, meningitis, migraine, multiple sclerosis, myelopathy, or a neurodegenerative disease (including, for example, Alzheimer's disease, Amyotrophic lateral sclerosis, Friedreich ataxia, Huntington's disease, Lewy body disease, Parkinson's disease, and Spinal muscular atrophy). In some embodiments, the disease, disorder or condition of the nervous system is a neural degenerative condition, optionally wherein the neural

degenerative condition involves inflammation and or activation of reactive oxygen species (ROS).

In some embodiments, the MSCs are capable of homing and/or trafficking to the CNS. For example, the MSCs may be capable of homing and/or trafficking to the brain, preferably wherein the MSCs are capable of homing to and/or trafficking into the brain through an intact blood-brain barrier (BBB). In some embodiments, the homing and/or trafficking of MSCs to the brain may be determined by a suitable assay, such as by including a radiolabel in the MSCs that may be detected using a cerebral scan. In some embodiments, the MSCs preferentially home and/or traffic to the CNS as compared with off-target sites, such as the lung, spleen, testes and/or sciatic nerve. In some embodiments, the MSCs home and/or traffic exclusively to the CNS. Preferential homing and/or trafficking may be assessed using a suitable assay, such as the radiolabel describe above, and comparing the level detected in an intended site (such as the brain) with an unintended site (such as the lung, spleen, testes and/or sciatic nerve), and determining a ratio for preferential homing and/or trafficking.

In some embodiments, the MSCs are administered at a therapeutically effective amount, or in such a manner as to reach or maintain a therapeutically effective amount. For example, the MSCs may be administered at least once at a dose of between 1-5 million cells per kg of subject, for example between 2-4 million cells per kg of subject, preferably at a dose of 3 million cells per kg of subject. In some embodiments, the MSCs are administered at least once per dosage regime, for example at least twice per dosage regime, preferably at least 3 times per dosage regime. The administrations may happen on the same day, or may be days, weeks and/or months apart. In some embodiments, the MSCs are administered at 3 million cells per kg of subject as 3 separate administrations. The treatment course may be adjusted, and administrations performed "on demand" based on a clinical outcome (for example, based on the level of effect the treatment currently has on a symptom associated with the disease, disorder or condition).

In some embodiments, the MSCs are administered by intravenous (IV) or intraarterial (IA) administration, preferably by IA administration. In some embodiments, the MSCs are not administered directly to the nervous system. In some embodiments, the MSCs are not administered directly to the CNS. In some embodiments, the MSCs are not administered by intrathecal or intracranial administration.

In some embodiments, the isolated population of MSCs is comprised of at least 50% neural MSCs (e.g. OSCAR+ MSCs), for example at least 60%, 70%, 80%, 90%, 95%, 96%, 97%,

98%, or 99% neural MSCs (e.g. OSCAR+ MSCs), or comprise 100% neural MSCs (e.g. OSCAR+ MSCs). The percentage level of a surface marker may be determined using a suitable technique, such as flow cytometry. A population that comprises a higher percentage of a particular marker may be considered as enriched for that particular marker. For example, the isolation population of MSCs may be enriched for a preferred surface marker (e.g. OSCAR) as compared to an unsorted population, in which case the cells may be distinguishable from a natural population due to the higher percentage of the surface marker (e.g. OSCAR) versus a relevant control. Therefore, obtaining an isolated population of MSCs comprised of a particular percentage of MSCs with a preferred surface marker may involve enriching a starting population, for example by positively sorting the cells with a flow cytometer based on a surface marker(s) (e.g. OSCAR). Accordingly, the isolated population of MSCs may be enriched for expression of a surface marker (e.g. OSCAR) such that the population has at least 50% MSCs positive for the surface marker (e.g. OSCAR), for example at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 100% MSCs positive for the surface marker (e.g. OSCAR).

In preferred embodiments, the subject is a human subject.

In some embodiments, the MSCs further comprise at least one drug or agent. For example, the MSCs may be a delivery vehicle to the brain for at least one drug or agent that is loaded into the MSCs.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Murine study scheme.

Figure 2: Graphical representation of the number of axons at day 43. Cpt = cisplatin, * = 3 treatments of 3 million cells/kg- total 9 million cells/kg.

Figure 3: Graphical representation of the axonal diameter at day 43. Cpt = cisplatin, * = 3 treatments of 3 million cells/kg- total 9 million cells/kg.

Figure 4: Graphical representation of the mean of g-ratio at day 43. Cpt = cisplatin, * = 3 treatments of 3 million cells/kg- total 9 million cells/kg.

Figure 5: Histology images of sciatic nerve semithin cross sections stained with toluidine blue. Scale bar 10 µm.

Figure 6: qPCR probe and primers used in Example 2.

Figure 7: Graphical representation of the biodistribution of OSCAR+ MSCs in the brain. Biodistribution is measured in ng of human DNA detected in these organs, which would

only be attributable to the human OSCAR+ MSCs administered to the mice. Statistical significance assessed using Mann-Whitney U test (*<0.05; **<0.01).

Figure 8: Graphical representation of the biodistribution of OSCAR+ MSCs in the spleen and lungs. Biodistribution is measured in ng of human DNA detected in these organs, which would only be attributable to the human OSCAR+ MSCs administered to the mice. Statistical significance assessed using Mann-Whitney U test (*<0.05; **<0.01).

Figure 9: Graphical representation of the biodistribution of OSCAR+ MSCs in the testes and sciatic nerve. Biodistribution is measured in ng of human DNA detected in these organs, which would only be attributable to the human OSCAR+ MSCs administered to the mice. Statistical significance assessed using Mann-Whitney U test (*<0.05; **<0.01).

Figure 10: A histogram plot demonstrating the percentage of MSCs identified as OSCAR+ in the population of cells used in Examples 1 and 2, as determined by flow cytometry using an anti-OSCAR PE antibody. The left most peak (grey line) is the unstained control, whereas the peak that shifts to the right (black line) corresponds to the stained cells by P7 dated on the live single population.

DETAILED DESCRIPTION OF THE INVENTION

All publications, patents and patent applications cited herein, whether *supra* or *infra*, are hereby incorporated by reference in their entirety.

It is to be understood that different applications of the disclosed cell populations, uses, methods, pharmaceutical compositions and kits may be tailored to the specific needs in the art. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only and is not intended to be limiting.

The terms "population of cells", "population of MSCs", "MSCs" are used interchangeably throughout.

Methods of purifying, culturing and selecting MSC subpopulations with neonatal quality and adult tissue specificity are described in detail below. Examples disclosed herein relate to methods for collecting, purifying, isolating, expanding, differentiating, and maturing amniotic fluid-derived cells. The examples disclosed herein are not limited to collection of a certain type of amniotic-derived cell and the technologies disclosed herein are broadly applicable to different cells and tissues. Amniotic fluid may be collected to produce term amniotic fluid (TAF) and processed according to the methods described in US Patent Application No. 14/776,499 (corresponding to US2016/0030489), the entire content of which is incorporated by reference.

The provision of TAF, removal of particulate material from the TAF to obtain purified TAF cells, adherence selection, and passaging of the TAF adherence cells may be in accordance with WO 2021/076042 A1 and/or WO 2021/076043 A1, the entire contents of which are incorporated by reference.

Purification

Term amniotic fluid (TAF) is purified by filtering term amniotic fluid to remove vernix. Although the term 'term amniotic fluid' is employed here and elsewhere in the present disclosure, it is understood that methods, processes, and devices of the present disclosure may be applied to all amniotic fluids and not just term amniotic fluid. Term amniotic fluid may be amniotic fluid collected at term caesarean section deliveries using, for example, a closed catheter-based system. For the purposes of the present description, 'term amniotic fluid' may be amniotic fluid collected at planned cesarean sections after 37 completed weeks of pregnancy or later, or at planned cesarean section close to term, for example after 36 completed weeks of pregnancy. Preferably, term amniotic fluid is taken at planned caesarean sections during week 37 of pregnancy or later.

The amniotic fluid contains amniotic cells originating from the fetus or the amniotic sac such as mesenchymal stem cells (MSCs). The amniotic fluid also contains other materials chafed off the skin such as hair and vernix. Material other than the amniotic cells are here referred to as particulate matter and may also comprise meconium, blood clots, etc. Particulate matter may be considered as anything larger than 20 µm. For the purposes of filtering, it may be particularly advantageous to treat anything larger than 30 µm or even 50 µm as particulate matter. Optionally, anything larger than the targeted amniotic cells may be treated as particulate matter. The amniotic fluid thus generally contains a mixture of amniotic cells and particulate matter.

Removing particulate material from the TAF to obtain purified TAF cells may be done by applying any known method in the art such as filtration, centrifugation, etc. The TAF may be filtered through a filter having a pore size at or above 20 µm. The filter may be made from any synthetic material including but not limited to cellulose acetate, cellulose nitrate (collodion), polyamide (nylon), polycarbonate, polypropylene and polytetrafluoroethylene (Teflon).

Adherence Selection

Various terms known to one skilled in the art have been and will be used throughout the specification, for example, the terms "express, expression, and/or expressing" in the

context of a cell surface marker are meant to indicate the presence of a particular marker on the surface of a cell, said surface marker having been produced by the cell. Surface marker expression may be used to select between different cell populations, for example, positively selecting for surface marker expression indicates the selection of a cell population that more strongly expresses a particular surface marker as compared to another cell population. Conversely, negatively selecting for cell surface marker expression indicates the selection of a cell population that more weakly expresses a particular surface marker as compared to another cell population.

As explained above and elsewhere in the specifications, TAF contains various progenitor cell types. In certain examples, particular progenitor cell types may be isolated and propagated via adherence selection. For example, a vitronectin substrate, Synthemax (Merck, CORNING®, Synthemax®, II-SC SUBSTRATE, CLS3535-1EA) may be used as a coating to create a more *in vivo*-like environment for stem cell culture, thereby limiting maturation of the TAF-derived progenitor cells and maintaining plasticity. Synthemax is an animal-component free, synthetic, flexible vitronectin-based peptide substrate for serum or serum-free expansion of human progenitor/stem cells and other adult stem cell types. One of skill in the art will understand that the vitronectin-based peptide substrate may include a portion of a vitronectin protein, such as a particular peptide sequence of vitronectin. Alternatively, intact vitronectin protein may be used. Synthemax vitronectin substrate offers a synthetic, xeno-free alternative to biological coatings and/or feeder cell layers commonly used in cell culture and known in the art. Briefly, standard tissue-culture treated flasks may be coated with about 0.2 mL Synthemax/cm^2 at 10 µg/mL giving a surface density of 2 µg/cm^2, and incubated at 37°C for about 1h, 1.5h, 2h, 4h, 8h, or more than 8h or at room temperature for about 2h, 1h, 4h, 8h or more than 8h with surplus solution optionally being removed and replaced. In certain examples, Synthemax may be coated at a surface density of about: 1 to 5 µg/cm^2, such as 2 µg/cm^2, 1 to 10 µg/cm^2, 1.5 to 4 µg/cm^2, 1 to 3 µg/cm^2, or about 1.5 to 2.5 µg/cm^2.

In other embodiments, adherence selection can be performed using a surface coated with, for example, Collagen, Fibronectin. Alternatively, adherence selection can be performed using an uncoated surface comprising a tissue-culture treated plastic.

Cells purified from TAF fluid may be gently re-suspended in prewarmed xeno-free cell culture media, with the cell suspension is then added to the Synthemax-coated flasks. Media may be changed at various times after addition to the flasks, for example, after about: 2h to 168h, 12h to 96h, 24h to 72h, 36h to 60h, 42h to 56h, or 48h, and then subsequently changed about: every day, every other day, every third day, every fifth day,

once a week, once every two weeks or about less than once every two weeks. Through repeated removal of spent medium, the non-attached cells may be removed, thereby selecting the MSCs by their affinity for attachment to the Synthemax-treated surface. The cells may be cultured for a period of time, such as about, for example, 4d, 7d, 10d, 11d, 12d, 13d, 14d, 18d, 21d, 28d or longer than 21d. Optionally, the cells may be cultured under hypoxic conditions: hypoxia priming may alter cell metabolism during expansion, increase resistance to oxidative stress, and thereby improve the engraftment, survival in ischemic microenvironments, and angiogenic potential of transplanted MSCs. After culturing, the P0 colonies (Colony forming Units - CFUs) that have formed may be dissociated and pooled. After pooling, the remaining cells may be predominantly non-tissue specific MSCs. In certain examples, the pooled P0 cells may be gently re-suspended in pre-warmed xeno-free cell culture media and re-plated on tissue-culture treated flasks without Synthemax for passaging. The pooled cells may be seeded at a seeding density of from between about: 100 to 10000 cells/cm^2, 500 to 8000 cells/cm^2, 1000 to 5000 cells/cm^2, or about 2000 to 4000 cells/cm^2. The media may be changed about every 1d, 2d, 4d, or more than four days. After a period of time, such as about 2d, 4d, 7d, or more than 7d, the cells may be dissociated and harvested. Further selective MSC isolation may be achieved as described below.

Identification of Markers

When comparing the genetic expression profiles of TAF MSCs and adult-type MSCs derived from adipose tissue or bone marrow by RNAseq, TAF MSCs tend to express more of some genes present in adult-type MSCs and less of others. Identification of both positive and negative TAF MSC specific neonatal cell-surface markers can allow for sorting of the MSCs with neonatal quality from those that have differentiated further and are of less importance as progenitor cells using e.g. ligands such as antibodies and aptamers or other selection techniques.

The cell surface markers distinguishing tissue relevant cells from other MSCs may be elucidated via a bioinformatics process utilizing a tissue-specificity score algorithm (as described in more detail in WO 2021/076042 A1 and WO 2021/076043 A1 *supra*, see Figure 14 therein). Tissue-specificity may be measured as a combination of two components: a 'tissue transcriptional similarity' also known as a similarity score and a "tissue-specific gene expression program" also known as a gene set score. In certain examples, the similarity score may be an Average Spearman correlation to each MSC tissue reference sample (for example a fetal lung MSC sample). In examples, the gene set score may be the average expression of genes in a tissue-specific gene set. After normalizing the similarity and gene set scores using a Z-transform to convert the input

values, which is a sequence of real or complex numbers, into a complex frequency-domain representation, then combining them assigning equal weight to each score and transforming combined values using a Z-transform, the resulting output is an MSC tissue specificity score. The MSC tissue-specificity score measures the relative tissue-specificity among the input samples by measuring how many standard deviations a sample is more or less specific to a given tissue compared to the average input sample. For example, an MSC tissue-specificity score may indicate how much more a clone sample appears to have a tissue specific phenotype, such as a lung phenotype, as compared to an average clone. Such an approach allows for identification of the top X% percentile scores using a normal distribution function, effectively the top X% of clones that are most tissue-specific to the relevant tissue.

In one example, for a given tissue, tissue-prioritized clones can be defined as any clone belonging to the top X% percentile score, where X is any percentage within a range having a lower end from about 0.1 to 25, such as about 1, 5, 10, 15 and 20, and an upper end from about 30 to 75, such as about: 35, 40, 45, 50, 55, 60, 65 or 70. Having prioritized tissue-specific clones, candidate surface marker genes may then be identified. For each tissue, two groups may be defined: tissue-prioritized and tissue-distal. A suitable analysis program may be used to make this determination, for example DEseq2 from Bioconductor.org. The tissue-prioritized group may include clones with a score in the top 15% percentile. The tissue-distal group may include clones in the bottom Y% percentile in which Y is any percentage within the range having a lower end from about 25 to 70, such as about: 30, 35, 40, 45, 50, 55, 60 or 65 and an upper end from 75 to 99.9, such as about: 80, 85, 90, 95 or 99. Figure 16 of WO 2021/076043 A1 shows an example of such analysis on kidney tissue. Next, differentially expressed genes between the tissue-prioritized and tissue-distal groups may be identified. Finally, the differential expression results may be annotated with surface marker gene information.

In certain examples, to identify tissue-specific cell surface markers, surface marker genes with a more than a Z-fold increase, where Z is at least about: 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 5-fold, 8-fold, 10-fold, 12-fold, 15-fold or even more-fold increase in expression (log2FoldChange) in prioritized clones compared to an average clone and a Transcripts Per Kilobase Million (TPM) of more than about 500, such as more than about: 1000, 1500, 2000, 2500, 3000, 5000 or even higher may be selected to give the top tissue-specific marker candidates, such as approximately the top: 5, 10, 20, 30, 40, 50, 60, 70, 100 or more, for example such as those shown below in Table 1 and further described in more detail below. Suitable log2FoldChange and TPM values may vary even

further depending on tissue type specificities depending on the abundance/absence of good markers.

Marker-Based Selection

Amniotic fluid contains heterogenous cells in a homogenous fluid. Hence, a marker-based selection may be needed. One example of marker-based selection is via the use of Fluorescence activated cell sorting (FACS). FACS may be used to purify the cell population of TAF-MSCs, FACS allows for a very high purity of the desired cell population, even when the target cell type expresses very low levels of identifying markers and/or separation is needed based on differences in marker density. FACS allows the purification of individual cells based on size, granularity, and fluorescence. As will be understood by one of skill in the art, FACS may be used to select for certain cell populations that express one cell surface marker more than another cell population and vice-versa. In some examples of methods of purification, bulk methods of purification such as panning, complement depletion and magnetic bead separation, may be used in combination with FACS or as an alternative to FACS. In brief, to purify cells of interest via FACS, they are first stained with fluorescently-tagged monoclonal antibodies (mAbs), which recognize specific surface markers on the desired cell population. Negative selection of unstained cells may also allow for separation. For GMP production of cells according to some examples, FACS may be run using a closed system sorting technology such as MACSQuant® Tyto®. Samples may be kept contamination-free within the disposable, fully closed MACSQuant Tyto Cartridge. Further, filtered air may drive cells through a microchannel into the microchip at very low pressure (< 3 PSI). However, before entering the microchannel, potential cell aggregates may be held back by a filter system guaranteeing a smooth sorting process. The fluorescence detection system may detect cells of interest based on predetermined fluorescent parameters of the cells. Based on their fluorescent and scatter light signatures, target cells may be redirected by a sort valve located within the microchannel. For certain examples of methods of purification, the success of staining and thereby sorting may depend largely on the selection of the identifying markers and the choice of mAb. Sorting parameters may be adjusted depending on the requirement of purity and yield. Unlike on conventional droplet sorters, cells sorted by the MACSQuant Tyto may not experience high pressure or charge and may not get decompressed. Therefore, such a gentle sorting approach may result in high viability and functionality of cells. Alternatively, other marker-based selection techniques may be known to the skilled person and employed here. These include, but are not limited to, Magnetic-activated cell sorting, Microfluidic based sorting, Buoyancy activated cell sorting, mass cytometry etc.

Tissue Specific Cells and Usage (Neural TAF MSCs)

As explained above, analysis of RNAseq data from TAF-MSC clones, adult and neonatal MSC reference material as well as fetal fibroblasts and publicly available expression datasets may be used to identify and characterize TAF-MSC cells. For example, sub-populations of TAF-MSCs may be established by clustering their expression data (RNAseq) with neonatal reference samples. The sub-population described herein are neural TAF MSCs (also referred to herein as neural MSCs or as defined by particular surface markers in Table 1).

A number of surface markers of interest have previously been identified that are associated with neural TAF cells, and the isolation and preparation of neural TAF MSCs, has been described in WO 2021/076042. The neural TAF MSC surface markers identified in Table 1 may have at least a 3-fold increase in expression on prioritized clones compared to the average TAF-MSC clone (optionally with Transcripts Per Kilobase Million (TPM) threshold > 500). Moreover, as the number of different MSC-subtypes in TAF is limited, the selection of the tissue specific MSC may be done by firstly characterization, thereafter a stepwise negative selection/sorting of the material by taking into account the combined (multivariate) surface marker profile of the different tissue specific MSCs. One of skill in the art will understand that any such combination of these surface markers may be used for identifying and isolation of neural TAF cells from the general population of TAF-derived cells and/or TAF-MSC cells. In some examples, the below non-exclusive list of surface markers may be more highly expressed on the surface of neural-TAF cells as compared to other cell types, such as other TAF-derived cells and/or TAF-MSC cells.

Table 1: Neural TAF MSC markers.

1.	**OSCAR**	osteoclast associated Ig-like receptor;
2.	**HAVCR1**	hepatitis A virus cellular receptor 1;
3.	**ACKR3**	atypical chemokine receptor 3;
4.	**C3**	complement C3
5.	**SIRPB1**	signal regulatory protein beta 1;
6.	**SLC6A6**	solute carrier family 6 member 6;
7.	**CCKAR**	cholecystokinin A receptor;
8.	**TNFSF10**	TNF superfamily member 10;
9.	**CLSTN2**	calsyntenin 2;
10.	**TENM2**	teneurin transmembrane protein 2;
11.	**SFRP1**	secreted frizzled related protein 1;
12.	**PIK3IP1**	phosphoinositide-3-kinase interacting protein 1;
13.	**SCNN1D**	sodium channel epithelial 1 delta subunit;

14.	**CLDN11**	claudin 11;
15.	**ALDH3B1**	aldehyde dehydrogenase 3 family member B1; and/or
16.	**ITGB4**	integrin subunit beta 4

Preferably, the population of cells is selected based on OSCAR. Such a selection may result in the population being enriched for the OSCAR marker, for example following flow sorting using an OSCAR antibody. By enriching for the OSCAR marker, the OSCAR+ MSCs may be distinguished from unsorted MSCs based on the percentage of cellular population harbouring the OSCAR marker.

By "neural MSCs", we include the meaning that the MSCs have been sorted based on one or more surface marker listed in Table 1. For example, OSCAR+ MSCs are a form of neural MSCs.

The term "OSCAR+ MSCs" is used interchangeably herein with "OSCAR+ TAF MSCs" and "OSCAR+ cells". These terms include the meaning of mesenchymal stem cells (or cells having characteristic features and/or properties known in the art to be associated with MSCs) that have been enriched for expression of the surface marker OSCAR. By "enriched", we include the meaning that the cells have been isolated based on the surface marker (e.g. OSCAR), or selected from a population of cells to positively express the surface marker (e.g. OSCAR). For example, a population of MSCs (e.g. TAF MSCs) may be a mixed population in which some cells express the surface marker OSCAR, and some cells do not express the surface marker OSCAR, in which case obtaining a population of isolated OSCAR+ MSCs means that the OSCAR marker has been used to obtain the cells that express this marker. By selecting these cells, it is possible to enrich (i.e. expand the proportion of positive cells relative to negative cells) OSCAR+ MSCs within a population. Given that the OSCAR marker would not be expected to be present on every MSC (including MSCs sourced from TAF), it is thus possible to distinguish an OSCAR-sorted population from an unsorted population.

As will be understood by one of skill in the art, suitable combinations of the markers listed in Table 1 may be used to separate neural TAF MSCs from TAF MSCs by selecting for specific markers from Table 1 or combinations of two, three, four, five, six or more markers from Table 1. In certain examples, neural TAF MSCs can be more specifically identified by identifying a combination of stronger expression, such as 3-fold or more stronger expression (optionally with TPM threshold > 500) of any combination of the foregoing markers, e.g., HAVCR1 and/or ACKR3 and/or OSCAR and/or C3 and/or SIRPB1 and/or SLC6A6 as compared to TAF-MSCs. When using combinations of markers, identification

may be achieved with a lower threshold of stronger expression, such as 2-fold or more or a higher threshold such as 6-fold or more, 8-fold or more, or 12-fold or more expression of each of the markers. In addition, those skilled in the art will also recognize that combinations including both negative and positive markers, such as at any of the thresholds described above, can also be effective to more specifically isolate neural TAF MSCs.

In some embodiments, the isolated TAF MSCs have been pre-sorted or enriched to contain markers of interest using the techniques described herein. For example, the selecting step may enrich the population of TAF MSCs to comprise at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% neural TAF MSCs (e.g. OSCAR+ MSCs). For example, in the method of making an isolated population of MSCs, the MSCs obtained may be a population comprised of at least 50% neural MSCs (e.g. OSCAR+ MSCs), for example, at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% neural MSCs (e.g. OSCAR+ MSCs).

In some embodiments, the remaining percentage of cells may be a mixture with another type of MSC, TAF MSC (for example, as described in WO 2021/076042 A1), and/or with unsorted TAF MSCs.

In some embodiments, the isolated population of MSCs have been passaged multiple times. For example, the isolated population of MSCs may have been passaged 1, 2, 3, 4, 5, 6, or more times.

The MSCs described herein are capable of homing and/or trafficking (or to have the capability of homing and/or trafficking) to the CNS, for example the brain. By "capable of homing" and "capable of trafficking", we include the meaning that the cells, upon administration to a subject/recipient (e.g. by IV or IA administration), transport to the CNS. For example, the cells can extravasate from the circulation and into the CNS. By "homing", we include the meaning that the cells, upon administration, move from the site of administration and towards the intended site (i.e. the CNS), for example to the blood vessels outside of the brain parenchyma, optionally wherein the cells preferentially accumulate closer to the intended site than a non-intended site. By "trafficking", we include the meaning that the cells, upon administration, move from the site of administration and into the intended site (i.e. the CNS, for example by passing the BBB into the brain), optionally wherein the cells preferentially accumulate within the intended site, for example at a higher rate than they accumulate within a non-intended site.

In particular, the cells are capable of homing to an intact BBB and/or trafficking into and passing beyond an intact BBB. The BBB is a highly selective semipermeable border of endothelial cells that prevents solutes in the circulating blood from non-selectively crossing into the extracellular fluid of the CNS. The BBB is formed by endothelial cells of the capillary wall, astrocyte end-feet ensheathing the capillary, and pericytes embedded in the capillary basement membrane. This system allows the passage of some small molecules by passive diffusion, as well as the selective and active transport of various nutrients, ions, organic anions, and macromolecules such as glucose and amino acids that are crucial to neural function. However, the BBB restricts the passage of pathogens, the diffusion of solutes in the blood, and large or hydrophilic molecules into the cerebrospinal fluid. The barrier also restricts the passage of peripheral immune factors, like signalling molecules, antibodies, and immune cells, into the CNS, thus insulating the brain from damage due to peripheral immune events.

Homing and/or trafficking of MSCs within a subject can be assessed using techniques known to the skilled person. For example, the MSCs may be modified to include a radiolabel (for example, an antibody conjugated to a radiolabel, wherein the antibody has specificity for a target on the cell surface of the MSCs). Such a radiolabel can be tracked *in vivo* with PET or SPECT imaging, as described in, for example, Gawne *et al.*, 2022. Accordingly, the proportion of MSCs homing and/or trafficking to the CNS may be quantified. The same technique may be used to assess the level of homing and/or trafficking to sites other than the CNS (for example, the lungs, spleen, testes and/or sciatic nerve), and a relative level of higher MSC homing and/or trafficking to the CNS (e.g. the brain) can be expressed as compared with non-CNS sites (e.g. the lungs, spleen, testes and/or sciatic nerve). Therefore, in some embodiments, the homing and/or trafficking of the MSCs to the CNS (e.g. the brain) is higher than the homing and/or trafficking of the MSCs to the lungs, spleen, testes and/or sciatic nerve. In preferred embodiments, the MSCs home and/or traffic exclusively to the CNS (e.g. the brain).

By "intact" or "substantially intact", we include the meaning that BBB does not have a level of adverse pathology or damage that disrupts the non-selective movement of solutes or cells from the circulation and into the brain. An intact BBB may also be considered a healthy BBB, a fully intact BBB and/or a functional BBB. The skilled person is aware of means to assess an intact BBB, for example as based on osmotic gradients across the endothelium at the BBB. An intact BBB typically has very low paracellular permeability and high trans-endothelial electrical resistance (TEER), and brain endothelial cells present very low rates of vesicle trafficking, limiting the transcytosis transport further contributing to a functional BBB (Ayloo *et al.*, 2019). Integrity of the BBB may be measured, for

example, by using magnetic resonance imaging (Kassner and Thornhill, 2011). Alternatively, or additionally, the integrity of the BBB may be assessed using a tracer leakage assay, variations of which are described in O'Brown *et al.*, 2018 (see, for example, the section entitled "Defining BBB permeability").

The MSCs or pharmaceutical composition thereof may be delivered in a therapeutically effective amount. By "therapeutically effective amount", "effective amount", "therapeutic effect" or "therapeutically effective", it is meant that a given substance is administered to a subject suffering from a condition, in an amount sufficient to cure, alleviate or partially arrest the condition or one or more of its symptoms. Such therapeutic treatment may result in a decrease in severity of disease symptoms, or an increase in frequency or duration of symptom-free periods. Effective amounts for a given purpose and a given agent will depend on the severity of the disease or injury as well as the weight and general state of the subject. This may be a predetermined quantity of active antibody calculated to produce a desired therapeutic effect in association with the required additive and diluent, i.e. a carrier or administration vehicle. Further, it is intended to mean an amount sufficient to reduce or prevent a clinically significant deficit in the activity, function, and response of the host. Alternatively, a therapeutically effective amount is sufficient to cause an improvement in a clinically significant condition in a host. As is appreciated by those skilled in the art, the amount of a compound may vary depending on its specific activity. Suitable dosage amounts may contain a predetermined quantity of active composition calculated to produce the desired therapeutic effect in association with the required diluent. A therapeutically effective amount can be determined by the ordinary skilled medical or veterinary worker based on patient characteristics, such as age, weight, sex, condition, complications, other diseases, etc., as is well known in the art.

As used herein, the terms "treat", "treatment", "treating", or "amelioration" when used in reference to a disease, disorder or medical condition, refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent, reverse, alleviate, ameliorate, inhibit, lessen, slow down or stop the progression or severity of a symptom or condition. The term "treating" includes reducing or alleviating at least one adverse effect or symptom of a condition. Treatment is generally "effective" if one or more symptoms or clinical markers are reduced. Alternatively, treatment is "effective" if the progression of a disease, disorder or medical condition is reduced or halted. That is, "treatment" includes not just the improvement of symptoms or markers, but also a cessation or at least slowing of progress or worsening of symptoms that would be expected in the absence of treatment. Also, "treatment" may mean to pursue or obtain beneficial results or lower the chances of the individual developing the condition even if the

treatment is ultimately unsuccessful. Those in need of treatment include those already with the condition as well as those prone to have the condition or those in whom the condition is to be prevented.

In some embodiments, the MSCs cross the BBB (preferably an intact BBB) to exert the therapeutic effect or treat or prevent the disease, disorder or condition of the nervous system. Therefore, the MSCs may be suitable for crossing an intact BBB as described herein.

"Beneficial results" or "desired results" may include, but are in no way limited to, lessening or alleviating the severity of the disease condition, preventing the disease condition from worsening, curing the disease condition, preventing the disease condition from developing, lowering the chances of a patient developing the disease condition, decreasing morbidity and mortality, and prolonging a patient's life or life expectancy. As non-limiting examples, "beneficial results" or "desired results" may be alleviation of one or more symptom(s), diminishment of extent of the deficit, stabilised (i.e., not worsening) state of a pathology, and amelioration or palliation of symptoms associated with the pathology.

A therapeutically or prophylactically significant reduction in a symptom is, e.g., at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 125%, at least about 150% or more in a measured parameter as compared to a control or non-treated subject or the state of the subject prior to administering MSCs. Measured or measurable parameters include clinically detectable markers of disease, for example, elevated or depressed levels of a biological marker, as well as parameters related to a clinically accepted scale of symptoms or markers for fibrosis and/or inflammation. It will be understood, however, that the total daily usage of the compositions and formulations as disclosed herein will be decided by the attending physician within the scope of sound medical judgment. The exact amount required will vary depending on factors such as the type of disease being treated, gender, age, and weight of the subject.

An advantage of the MSCs described herein is the preferential homing and/or trafficking to the CNS, such as the brain, as compared with off-target sites such as the lungs, spleen, testes and/or sciatic nerve. By having preferential homing and/or trafficking to the brain, for example, a lower dose of the cells (and, optionally, any drugs or agents loaded into the cells) is required to mediate beneficial effects in the brain. Alternatively, or additionally, the preferential homing and/or trafficking to the CNS (e.g. the brain) may be

as compared with a negative control, such as a sham control or a vehicle control, or as compared with an alternative source of MSC than TAF MSCs selected/enriched for a neural surface marker (e.g. OSCAR). By "preferential targeting", we include the meaning that the MSCs are detectable in an intended site (i.e. the CNS) at a higher level than a non-intended site (e.g. the lungs, spleen, testes and/or sciatic nerve).

Each one of the conditions of the nervous system described herein are well-known, and the symptoms and diagnostic markers are well described, as are the therapeutic agents used to treat those conditions. Accordingly, the symptoms, diagnostic markers, and therapeutic agents used to treat the above-mentioned conditions would be known to those skilled in medicine.

The MSCs described herein have several beneficial properties, such as the ability to mediate inflammation and/or fibrosis. However, the MSCs may also be used as a delivery vehicle to get at least one drug or agent into the brain, via an intact BBB. This specialised form of MSCs is therefore a platform technology that may be adapted to various diseases, disorders or conditions of the nervous system, based on the required, available or preferred drug or agent.

In some embodiments, the MSCs are administered in combination with at least one drug or agent, sequentially, simultaneously and/or subsequently. For example, the further agent may be administered as part of the pharmaceutical composition comprising the MSCs, and/or by loading the MSCs with at least one drug or agent. In some embodiments, the drug or agent is selected from the group consisting of nervous system drugs, anti-inflammatory agents, immunosuppressive agents, anti-tumour agents/drugs and any combinations thereof. The term "nervous system drugs" indicates that the agent or drug reduces or prevents a pathology associated with the nervous system. The term "anti-inflammatory agent" indicates that the agent or drug reduces or prevent an immune response that causes inflammation. The term "immunosuppressive agents" indicates that the agent or drug blocks or reduces the activity of an immune response, which may be a proinflammatory or anti-inflammatory response. The term "anti-tumour agent/drug" indicates that the agent or drug has activity against cancer, for example a chemotherapeutic agent. An agent or drug may fall within the definition of any one or more of these terms, and so the terms may be used herein interchangeably. If administered simultaneously, the administration of the MSCs and the further agent(s) may be as separate administrations at the same time, and/or as a single administration of the MSCs loaded with the further agent(s).

In some embodiments, the MSCs or pharmaceutical composition comprising MSCs are administered more than once. For example, the MSCs may be administered at least twice or at least 3 times. For example, administration may occur 4, 5, 6, 7, 8, 9, 10 or more times. These administrations may be part of a regime (i.e. dosage regime). The term "regime", as used herein is synonymous with regimen or regiment. The MSCs or pharmaceutical compositions described herein may be administered as a dosage regimen. By "dosage regime" we include the meaning that the population of cells are administered in steps, wherein multiple steps form a regime.

In some embodiments, the dosage regimes described herein can be repeated as many times as necessary in a particular subject. For instance, this dosage regime can be employed each and every time the population of cells is administered to the subject. In some embodiments, the exact format of the dosage regime (in terms of timing and amounts of doses) may be varied between repeat administrations to the subject. The advantage of using the dosage regimes described herein repeatedly is that it reinforces the therapeutic effects.

However, as a person skilled in the art will appreciate, repeat dosing could also utilise higher or lower total doses as guided by patient tolerability. Analogous flat dosing-based, or receptor-occupancy guided, dosing regimens could be used.

In some embodiments, the multiple administrations of MSCs or pharmaceutical composition comprising MSCs occur at least 1 day, 2 days, 3 days, 4 days, 5 days, or 6 days apart. In some embodiments, the multiple administrations of MSCs or pharmaceutical composition comprising MSCs occur at least 1 week, 2 weeks, 3 weeks, or 4 weeks apart. In some embodiments, the multiple administrations of MSCs or pharmaceutical composition comprising MSCs occur at least 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months or more apart. The multiple administrations of the MSCs or pharmaceutical composition comprising MSCs may occur as any combination of the days, weeks, or months described above.

As used herein, the term "administering" or "administration", refers to the placement of MSCs or a pharmaceutical composition as disclosed herein into a subject by a method or route which results in at least partial localisation of the agents or composition at a desired site. "Route of administration" may refer to any administration pathway known in the art, including but not limited to oral, topical, aerosol, nasal, via inhalation, anal, intra-anal, peri-anal, transmucosal, transdermal, parenteral, enteral, or local. "Parenteral" refers to

a route of administration that is generally associated with injection, including intratumoral, intracranial, intraventricular, intrathecal, epidural, intradural, intraorbital, infusion, intracapsular, intracardiac, intradermal, intramuscular, intraperitoneal, intrapulmonary, intraspinal, intrasternal, intrathecal, intrauterine, intravascular, intravenous, intraarterial, subarachnoid, subcapsular, subcutaneous, transmucosal, or transtracheal. Via the parenteral route, the agent or composition may be in the form of solutions or suspensions for infusion or for injection. Via the enteral route, the agent or composition can be in the form of capsules, gel capsules, syrups, suspensions, solutions, emulsions, or lipid vesicles or polymer vesicles allowing controlled release. Via the topical route, the agent or composition can be in the form of aerosol, lotion, cream, gel, ointment, suspensions, solutions or emulsions.

In some embodiments, the MSCs or pharmaceutical composition comprising MSCs are administered intravenously and/or intraarterially. In a preferred embodiment, the MSCs are administered intraarterially. Given that the MSCs described herein are capable of homing and/or trafficking to the CNS, and even to a brain with an intact BBB, it is not necessary to administer the cells intracranially or intrathecally to bypass the BBB. Therefore, in some embodiments, the MSCs are not administered directly to the CNS. For example, the MSCs are not administered intracranially or intrathecally.

In some embodiments, the population of cells is formulated and/or adapted for delivery by a route selected from the group comprising intravenous (i.e. "i.v" or "IV") and intraarterial (i.e. "i.a." or "IA") administration. Preferably, the population of cells is formulated and/or adapted for intraarterial delivery. Methods and formulations for IV or IA administration of a population of cells are well known in the art. In the present invention, any type of IV or IA administration may be used, such as injection or infusion.

The term "subject", (which herein is used interchangeably with "patient" and "recipient") includes any animal, including a human, that is in need of treatment with a population of cells as described herein. The subject or patient may be mammalian or non-mammalian. Preferably, the subject is mammalian, such as a horse, or a cow, or a sheep, or a pig, or a camel, or a dog, or a cat. Most preferably, the mammalian patient is a human.

Preferably, the subject is one that has been diagnosed as having a condition of the nervous system, or that has been identified as likely to have a condition of the nervous system, and/or that exhibits symptoms of a condition of the nervous system. By "exhibits", we include that the subject displays a symptom and/or a diagnostic marker associated with a condition of the nervous system (as listed herein), and/or the symptom and/or a diagnostic

marker can be measured, and/or assessed, and/or quantified. It would be readily apparent to the person skilled in medicine what the symptoms and diagnostic markers would be and how to measure and/or assess and/or quantify whether there is a reduction or increase in the severity of the symptoms, or a reduction or increase in the diagnostic markers; as well as how those symptoms and/or diagnostic markers could be used to form a prognosis for the condition of the nervous system. In further preferable embodiments, the subject is one that has been diagnosed as having a condition of the nervous system, or that has been identified as likely to have a condition of the nervous system, and/or that exhibits symptoms of a condition of the nervous system, wherein the subject still has an intact BBB, or at least substantially intact BBB, as described herein.

The invention also provides a kit. The kit may be for preparing a suitable population of MSCs. For example, the kit may include an agent for selecting MSCs based on a preferred surface marker, e.g. OSCAR, such that OSCAR+ MSCs may be enriched in a population. The kit may further comprise a reconstitution medium that is suitable for preparing the MSCs ready for administration to a recipient.

The kits of the invention may additionally comprise one or more other reagents or instruments which enable any of the embodiments mentioned above to be carried out. Such reagents or instruments include one or more of the following: suitable buffer(s) (aqueous solutions) and means to administer the population of cells (such as a vessel or an instrument comprising a needle). The kit may include instructions for performing a therapy or method as described herein.

The population of cells described herein, or provided in the kits of the invention, may be provided as a pharmaceutical composition (used interchangeably herein with "a composition") formulated together with at least one pharmaceutically acceptable carrier, excipient or further component such as at least one therapeutic and/or prophylactic ingredient. A "pharmaceutically acceptable carrier" as referred to herein, is any known compound or combination of known compounds that are known to those skilled in the art to be useful in formulating pharmaceutical compositions. The carrier may include one or more excipients or diluents. Pharmaceutical compositions of the invention can be placed into dosage forms, such as in the form of unit dosages. Pharmaceutical compositions include those suitable for any route of administration (as disclosed herein).

All of the features disclosed in this specification (including any accompanying exhibits, claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined in any combination, except combinations where at least some

of such features and/or steps are mutually exclusive. The disclosure is not restricted to the details of any foregoing examples. The disclosure extends to any novel one, or any novel combination, of the features disclosed in this specification (including any accompanying claims, abstract and drawings), or to any novel one, or any novel combination, of the steps of any method or process so disclosed.

Those skilled in the art will appreciate that in some examples, the actual steps taken in the processes illustrated or disclosed may differ from those shown in the figures. Depending on the example, certain of the steps described above may be removed, others may be added. For example, the actual steps or order of steps taken in the disclosed processes may differ from those shown in the figure. Depending on the example, certain of the steps described above may be removed, others may be added. Furthermore, the features and attributes of the specific examples disclosed above may be combined in different ways to form additional examples, all of which fall within the scope of the present disclosure.

Conditional language, such as "can", "could", "might", or "may", unless specifically stated otherwise, or otherwise understood within the context as used, is generally intended to convey that certain examples include, while other examples do not include, certain features, elements, or steps. Thus, such conditional language is not generally intended to imply that features, elements, or steps are in any way required for one or more examples or that one or more examples necessarily include logic for deciding, with or without user input or prompting, whether these features, elements, or steps are included or are to be performed in any particular example. The terms "comprising", "including", "having", and the like are synonymous and are used inclusively, in an open-ended fashion, and do not exclude additional elements, features, acts, operations, and so forth. Also, the term "or" is used in its inclusive sense (and not in its exclusive sense) so that when used, for example, to connect a list of elements, the term "or" means one, some, or all of the elements in the list. Likewise, the term "and/or" in reference to a list of two or more items, covers all of the following interpretations of the word: any one of the items in the list, all of the items in the list, and any combination of the items in the list. Further, the term "each", as used herein, in addition to having its ordinary meaning, can mean any subset of a set of elements to which the term "each" is applied. Additionally, the words "herein", "above", "below", and words of similar import, when used in this application, refer to this application as a whole and not to any particular portions of this application.

Conjunctive language such as the phrase "at least one of X, Y, and Z", unless specifically stated otherwise, is otherwise understood with the context as used in general to convey

that an item, term, etc. may be either X, Y, or Z. Thus, such conjunctive language is not generally intended to imply that certain examples require the presence of at least one of X, at least one of Y, and at least one of Z.

Language of degree used herein, such as the terms "approximately", "about", "generally", and "substantially" as used herein represent a value, amount, or characteristic close to the stated value, amount, or characteristic that still performs a desired function or achieves a desired result. For example, the terms "approximately", "about", "generally", and "substantially" may refer to an amount that is within less than 10% of, within less than 5% of, within less than 1% of, within less than 0.1% of, and within less than 0.01% of the stated amount. As another example, in certain examples, the terms "generally parallel" and "substantially parallel" refer to a value, amount, or characteristic that departs from exactly parallel by less than or equal to 15 degrees, 10 degrees, 5 degrees, 3 degrees, 1 degree, or 0.1 degree.

Various modifications to the implementations described in this disclosure may be readily apparent to those skilled in the art, and the generic principles defined herein may be applied to other implementations without departing from the spirit or scope of this disclosure. Thus, the disclosure is not intended to be limited to the implementations shown herein, but is to be accorded the widest scope consistent with the principles and features disclosed herein. Certain examples of the disclosure are encompassed in the claim set listed below or presented in the future.

All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising", "having", "including" and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not

limited to") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

The listing or discussion of an apparently prior published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge.

Preferences, options and embodiments for a given aspect, feature or parameter of the invention should, unless the context indicates otherwise, be regarded as having been disclosed in combination with any and all preferences, options and embodiments for all other aspects, features and parameters of the invention. Embodiments and features of the present invention are also outlined in the following items and illustrated by the following non-limiting examples.

EXAMPLES

Example 1 – Cisplatin-induced peripheral neuropathy

A cisplatin-induced peripheral neuropathy (CIPN) murine model was prepared by administration of cisplatin in three weeklong cycles. The cisplatin used was controlled to avoid risk of significant damage to the blood-brain barrier (BBB), such that the BBB would remain intact (i.e. not leaky to substances that would otherwise be precluded from the brain).

List of abbreviations

ABR	Auditory brainstem responses
Admin.	Administration
AP	Action potential
C	Celsius
CMAP	Compound muscle action potential
CIHL	Chemotherapy-induced hearing loss
CIPN	Chemotherapy-induced peripheral neuropathy
Cpt	Cisplatin
D	Day
EMG	Electromyography (electrophysiology of PNS)
g	Gram
IA	Intraarterial
IV	Intravenous
IP	Intraperitoneal
kg	Kilogram
mg	Milligram
min	Minutes
mL	Millilitre
mo	Month old
mpk	Milligram per kilogram
mV	Millivolts
NA	Not assessed
NaCl	Sodium chloride
NCV	Nerve conduction velocity
Nd	Not determined
PNS	Peripheral nervous system
RT	Room temperature
SC	Schwann cell
SEM	Standard Error of the Mean
vhc	Vehicle
vs.	Versus

The treatment schedule was as follows:

- First cisplatin cycle from day 3 to day 6;
- First administration of OSCAR+ MSCs on day 7;

- Second cisplatin cycle from day 16 to day 19;
- Second administration of OSCAR+ MSCs on day 20;
- Third cisplatin cycle from day 29 to day 32;
- Third administration of OSCAR+ MSCs on day 33.

The MSCs used were prepared by Amniotics AB by obtaining MSCs from term amniotic fluid (TAF) (as described herein and in WO 2021/076042 A1) and enriching the population based on positive selection of the surface marker, OSCAR, to about 42% OSCAR+ cells in the population (see Figure 10). These are referred to herein as OSCAR+ MSCs.

Cells were supplied as a frozen aliquot that has been stored at -80°C, which was thawed immediately before injection, ensuring the substance was not kept at RT for more than 30 minutes after thawing. For the cell concentration of 3 million cells/kg mouse, the compound cell concentration was of 0.6 million cells/mL. The injected volume was therefore: 30 gram mouse = 90 000 cells = 150 µL; 25 gram mouse = 75 000 cells = 125 µL; 20 gram mouse = 60 000 cells = 100 µL.

Chemical products information

Product	Supplier	Admin.	Reference	Frequency	Stock temp.	Dose	Max. admin. Volume
Ketamine 1000	Virbac	IP	#0359713 2111010	Anesthesia	4°C	100 mg/kg	10 mL/kg
Xylazine	Rompun	IP	#04007221 032311	Anesthesia	4°C	10 mg/kg	10 mL/kg
Isoflurane (vetflurane)	Virbac	Gas	#Vnr137317 GTIN 0359713 2002653	Anesthesia	RT	2.5%	NA
Cisplatin	Teva	IP	#3400956 103294	Once a day. Three cycles of 4 injections/ Cycle	4°C	3.5 mg/kg	10 mL/kg

Animals

Sex/Strain/Species:	C57BL6 Male 8 weeks old
Approximate weight at initiation of treatment	25.0 g ± 2.5
Animal number	10 mice per group Total: 40 mice

Number of groups	Group 1: Sham control (no cisplatin – negative control); Group 2: Cisplatin + vehicle administered intravenously (IV); Group 3: Cisplatin + 3 million cells/kg administered intravenously (IV) (3 injections of 3 million cells/kg = total 9 million cells/kg); and Group 4: Cisplatin + 3 million cells/kg administered intraarterially (IA) (3 injections of 3 million cells/kg = total 9 million cells/kg).
Animal identification	At the beginning of the study, animals were identified with a number on the tail. Each parameter was noted in the lab book.
Experimental time duration	43 days: Readouts measures at days 1&2 and days 42&43 Cisplatin treatment from day 3 to day 32 (three cycles of four injections/cycle) Plasma sampling at day 1 and day 43 Organs sampling at day 43 Treatment of groups 3 and 5 at day 7, day 20 and day 33, and treatment of groups 2 and 4 at day 33

Housing: Mice were housed in disposable makrolon cages (Innovive, M-BTM) with filter hoods, in a room where the air is continuously filtered, thereby avoiding contamination. During experiments, paired animals were caged at a constant temperature with a day/night cycle of 12/12 hours.

Diet and water: Animals received water (Innovive, M-WT-300) and nutrition (SAFE, A03) ad libitum.

Animal protocol is approved by the Animal Studies Committee of Languedoc Roussillon. This protocol and our laboratory procedures comply with French legislation, which implements the European Directives (Reference Number: D3417223, APAFIS#23920-2020020320279696 v3).

Animal health, mortality and clinical signs were examined every day to ensure that only animals in good health enter to the testing procedures and follow up the study.

The overall *in vivo* study scheme can be seen in Figure 1.

The organs sampled from the mice include the lung, testis, brain, spleen, and sciatic nerve, with 100 µL of plasma also taken, all of which was obtained on day 43.

<u>Intravenous MSC administration</u>

Application of heat to the whole animal or locally to the tail was to cause vasodilatation making vascular access easier. The tail vessels were dilated by placing the tail in warm

water (37°C), never exceeding 40-44°C range. The animal's body temperature never exceed 40°C for over 5 minutes. Animals were constantly monitored for signs of heat distress. The mouse was restrained so that its tail is accessible. A 22G needle was used to avoid damage to the MSCs during injection. The vein was located, the needle inserted by directing the needle into the vein with its bevel facing upward at an angle of approximately 20 degrees. The needle was inserted slowly. Once the vein's wall was penetrated, the needle's angle was decreased, and the needle directed cranially approximately 2 mm. Blood was aspirated into the needle's hub before making the injection. During material administration, the vein should blanch as fluid runs through it, and no material or swelling should be detectable at the injection site—this indicates injection is outside of the vein. The OSCAR+ MSCs were administered slowly to avoid vascular overload or rupture of the vein from excess pressure. Once the needle was removed, pressure was applied over the injection site by gently holding a piece of gauze over the injection site for approximately 30 seconds to prevent hematoma formation.

Femoral artery catheterization for intra-arterial compound administration

For femoral artery catheterization, mice were anesthetized using ketamine/xylazine mixture (100 mg/kg and 10 mg/kg respectively).

Cannulation of the femoral artery was performed under a dissection microscope (VWR, Radnor, PA). A 1 cm incision was made at the inguinal region on the right hind limb parallel to the femoral vascular bundle. The superficial fascia layers were carefully dissected to visualize the femoral artery and vein. After visualization of the branches of the femoral artery, cannulation of the femoral artery was performed proximal or distal to the superficial caudal epigastric artery. The inguinal fat tissue was carefully dissected from the neurovascular bundle using blunt dissecting forceps and haemostasis was achieved with brief pressure or cautery if needed. After careful isolation of femoral nerve from the bundle, the femoral artery was separated from the vein using fine dissecting forceps. A 6-0 silk suture (Ethicon, Blue Ash, OH) was used proximal to each cannulation site for the temporary ligation of the artery during procedure. After making a partial thickness incision in the artery wall using micro iris scissors (Fine Science, Foster City, CA), the 32-gauge intrathecal catheter (Harvard Apparatus, Holliston, MA) was guided into the artery. After proper insertion of the guide wire and subsequently, the overlaid plastic catheter, the wire was drawn back gradually as the catheter was advanced forward. At this stage, the distal suture was placed over the cannulated artery to secure the catheter inside the artery. Then the guide wire was completely removed, the catheter was fixed using silk suture and placed over the mouse neck for IA injections.

Sciatic nerve electrophysiology

Standard electromyography was performed on mice anesthetized with ketamine/xylazine mixture (100 mg/kg & 10 mg/kg respectively) following SOP-A15-V1. A pair of steel needle electrodes (AD Instruments, MLA1302) were placed subcutaneously along the nerve at the sciatic notch (proximal stimulation). A second pair of electrodes were placed along the tibial nerve above the ankle (distal stimulation). Supramaximal square-wave pulses, lasting 10 ms at 1 mA were delivered using a PowerLab 26T (AD Instruments). Compound muscle action potential (CMAP) was recorded from the intrinsic foot muscles using steel electrodes. Both amplitudes and latencies of CMAP were determined and exported in excel. The distance between the 2 sites of stimulation was measured alongside the skin surface with fully extended legs, and nerve conduction velocities (NCVs) were calculated automatically from sciatic nerve latency measurements using Excel.

Plasma sampling, sciatic nerve and organ sampling

For each animal, 1 mL of blood was sampled by cardiac puncture and collected in a tube containing heparin lithium as anticoagulant. Samples were centrifuged for 15 minutes at 1000 × g (or 3000 rpm) at 2-8°C within 30 minutes of collection. 500 µL of supernatant (plasma) was stored at -20°C before ELISA analysis.

After blood sampling, five animals per group were sacrificed by cervical dislocation. Then, the left leg skin was opened, and the sciatic nerve was sampled. The nerve was fixed in glutaraldehyde 2.5% and PFA 4% overnight at 4°C and stored in PHEM buffer for complementary histopathology analysis. Then, the temporal bone was opened, and the brain was sampled, transferred into an Eppendorf tube and immediately stored at -80°C. Finally, the abdominal cavity was opened, and the lungs, testis, spleen and contralateral sciatic nerve was sampled, transferred separately into Eppendorf tubes and immediately stored at -80°C.

Nerve histology analysis by toluidine blue staining

The left sciatic nerves of five animals per group were sampled and fixed with 4% PFA and 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) overnight at 4°C. After washing for 30 minutes in 0.2 M PBS buffer, the samples were incubated in sucrose 30% for 48 hours and then embedded in OCT at -20°C. Semithin cross sections were cut and stained with 0.5 % of toluidine blue + 1 % borax + 100 mL MilliQ water. The axonal diameter, number of myelinated motor axons and the myelin g-ration was quantified using automatic Image J g-ratio plug-in.

Study phases

Acclimation and clinical signs: Animals arrived on site 7 days before the experiment to allow optimal acclimation. Clinical signs and mortality were recorded daily. Body weight was determined twice a week. Each parameter was noted in the lab book.

Identification and randomization: At the beginning of the study, all animals were weighted and identified with a number on the tail. Each parameter or clinical signs was noted in the lab book. Randomization was performed using the body weight data before the in vivo study phase. The animals were distributed in the 5 groups. Then each cage (5 mice/cage) was labelled with a record card, indicating the corresponding group and animal identification.

Statistical analysis

Descriptive statistics by groups were expressed as mean ± SEM for continuous variables. Statistical significances were determined using 2-way ANOVA, followed by a Bonferroni multiple comparisons post hoc test, allowing comparisons between groups, assuming the normal distribution of the variable and the variance homoscedasticity. Statistical analyses were performed using GraphPad Prism version 5.02 for Windows, GraphPad Software, La Jolla California USA. A P value of less than 0.05 was considered significant.

Histology analysis of sciatic nerve

Sciatic nerve toluidine blue staining was performed to determine the efficacy of the sponsor cell treatment from a histological point of view.

As expected, strong histopathology of sciatic nerve was observed in the cisplatin+vehicle treated group characterized by a significant decrease of the number of axons and axonal diameter and an increase of g-ratio (decrease of myelin sheath diameter) at day 43 (*Figures 2 to 5*).

No statistical differences on the number of axons, axonal diameter and g-ratio were observed in any cell treated group (cisplatin+3 million cells/kg IV and cisplatin+3 million cells/kg IA) compared to the vehicle group. Moreover, all cisplatin treated groups presented an axonal diameter and number of axons statistically lower than the sham control group, and a g-ratio statistically higher than the sham control group. Taken together, these data suggest a lack of cell treatment efficacy on the sciatic nerve histopathology induced by chemotherapy at these experimental conditions.

Example 2 – Biodistribution of OSCAR+ MSCs

One major challenge in MSCs therapy is the recruitment of the MSCs to the target tissue. In this study, the biodistribution of the MSCs was evaluated by analyzing brain, lung, spleen, sciatic nerve, and testis tissue for human DNA. Given that a mouse model was used, and the MSCs are from a human origin, it is possible to assess homing and trafficking of the MSCs based on analysing the presence of human DNA in murine organs or tissues.

Method

DNA extraction was performed using DNeasy Blood & Tissue Kit (Qiagen cat.no:69504). The aim was to determine the presence of hAlu in mouse DNA. DNA was extracted from mouse tissue using DNeasy Blood and Tissue Kit according to manufacturer's instructions for DNA extraction from frozen tissue.

qPCR (a routine technique in the art) was performed using a standard reaction mix, wherein 19 µL of reaction mix was placed into each well to be used on a 96-well PCT plate. 4 µL of each standard and sample was placed into pre-assigned wells in the plate, the plate was covered and briefly centrifuged. qPCR was then performed.

Samples were obtained from the lungs, brain, testis, spleen, and sciatic nerve from mice.

Primers/probes used (see Figure 6): hAlu Probe ([FAM]TGAGGCAGGAGAATCGCTTGAACC [BHQ1]), hAlu forward Primer (TGGTGGCTCTCTCCTGTAAT), and hAlu reverse Primer (GATCTCGGCTCACTGCAAC).

Pipettes: NN pipettes. Calibrated once a year.

Preparation of samples: All samples were diluted with AE buffer.

Preparation of standards:

- Standards were prepared from MSCs obtained from Amniotics. Extracted mouse DNA was used as a diluent, 6 standards were prepared using a 10-fold dilution series.
- Standards were prepared with the following concentrations (Stock: 78.32 ng/mL):
    - STD 1: 25 ng/mL
    - STD 2: 2.5 ng/mL

- STD 3: 0.25 ng/mL
- STD 4: 0.025 ng/mL

Results

Interestingly, a surprisingly high level of accumulation occurred in the brain for both administration routes (*Figure 7*), with lower levels detected in the spleen and lungs (*Figure 8*), and, importantly, no significant levels observed in the testis or sciatic nerve (*Figure 9*). Trafficking to the lungs and spleen is not unexpected, given that the lungs have a filter effect on such cells, and the spleen is a common site for cells that have been degraded and/or phagocytosed.

These data demonstrate a preferential and surprising targeting of OSCAR+ MSCs to the brain, despite the intact BBB that would be expected in this mouse model. The targeting was particularly effective following IA administration compared with IV administration, although both routes of administration were successful.

Conclusion

The use of cisplatin in the treatment schedule was of a low enough dose and frequency to ensure no significant loss of integrity to the BBB in the mice. Cisplatin typically fails to cross the BBB, requiring modifications to enter the brain (such as coupling with a cell-penetrating peptide or encapsulating within a liposome, see Charest *et al.*, 2013).

In such a model of an intact BBB, there would be no expectation of MSCs homing or trafficking into the brain following IA or IV administration. However, the OSCAR+ MSCs (which are derived from a TAF source and enriched for the OSCAR marker) have the unusual property of being able to pass an intact BBB and enter the brain.

These data therefore reveal a preferential and highly significant recruitment of the neural marker specific MSCs to the brain in treatment groups with significant positive efficacy. Selective recruitment of the MSCs to the brain could increase cellular involvement in the brain tissue regeneration processes.

This study confirms the preferential recruitment of the neural marker specific MSCs to the CNS when the MSCs were administrated after each cisplatin treatment cycle (i.e. 3 times at a dose of $3x10^6$ cells/kg IV or IA).

This function of the OSCAR+ MSCs has several advantages associated with it. Firstly, the therapeutic window for various CNS-related pathologies is broadened, as the brain can be accessed at an earlier point in the pathology, such that minimal-to-no disruption of the BBB has occurred. Secondly, it presents a new therapeutic agent that can enter such a privileged biological site, without the need to adapt the therapeutic (for example, using a lipid-soluble mediator) for crossing the intact BBB. This function therefore provides the opportunity to target pathologies that do not disrupt the BBB. Thirdly, given that MSCs have been demonstrated to be interesting delivery vehicles, a platform such as the OSCAR+ MSCs presents the opportunity to load the cells with a drug or agent that has a mode of action within the brain, past the BBB. However, it will be appreciated that MSCs (such as the OSCAR+ MSCs) have several beneficial properties that may directly mediate pathology of the CNS in the brain, without need for a drug or agent that is loaded into the MSCs.

Furthermore, given that the endothelial barriers within the BBB are conserved across vertebrates (see O'Brown *et al.*, 2018), it is entirely plausible that the homing and trafficking observed in this murine model will extrapolate to that of the human. This is particularly likely given the structural similarities in, for example, the tight junction complexes between endothelial cells, which may be the site of extravasation for the MSCs into the brain (see O'Brown *et al.*, 2018).

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4. Ayloo S, Gu C. Transcytosis at the blood–brain barrier. Curr Opin Neurobiol. 2019;57:32–38.
5. Kassner and Thornhill, 2011, "Measuring the integrity of the human blood-brain barrier using magnetic resonance imaging", *Methods Mol. Biol.*, 686:229-45.
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CLAIMS

1. An isolated population of mesenchymal stem cells (MSCs) for use in treating or preventing a disease, disorder or condition of the nervous system in a subject in need thereof; wherein the MSCs express at least one surface marker selected from the group consisting of OSCAR, HAVCR1, ACKR3, C3, SIRPB1, SLC6A6, CCKAR, TNFSF10, CLSTN2, TENM2, SFRP1, PIK3IP1, SCNN1D, CLDN11, ALDH3B1 and ITGB4.

2. The isolated population of MSCs for use according to claim 1, wherein the surface marker is OSCAR.

3. The isolated population of MSCs for use according to any preceding claim, wherein the MSCs are obtainable from term amniotic fluid (TAF).

4. The isolated population of MSCs for use according to any preceding claim, wherein the disease, disorder or condition of the nervous system is of the central nervous system (CNS).

5. The isolated population of MSCs for use according to claim 4, wherein the disease, disorder or condition of the CNS is selected from the group consisting of: arachnoid cysts, brain tumours (including, for example, glioma or glioblastoma), catalepsy, encephalitis, epilepsy, neural infection, meningitis, migraine, multiple sclerosis, myelopathy, or a neurodegenerative disease (including, for example, Alzheimer's disease, Amyotrophic lateral sclerosis, Friedreich ataxia, Huntington's disease, Lewy body disease, Parkinson's disease, and Spinal muscular atrophy).

6. The isolated population of MSCs for use according to any preceding claim, wherein the MSCs are capable of homing and/or trafficking to the CNS.

7. The isolated population of MSCs for use according to claim 6, wherein the MSCs are capable of homing and/or trafficking to the brain.

8. The isolated population of MSCs for use according to claim 7, wherein the MSCs are capable of homing and/or trafficking into the brain though an intact blood-brain barrier.

9. The isolated population of MSCs for use according to any preceding claim, wherein the MSCs preferentially home and/or traffic to the CNS.

10. The isolated population of MSCs for use according to any preceding claim, wherein the MSCs are administered at least once at a dose of between 1-5 million cells per kg of subject, for example between 2-4 million cells per kg of subject.

11. The isolated population of MSCs for use according to claim 10, wherein the MSCs are administered at least once at a dose of 3 million cells per kg of subject.

12. The isolated population of MSCs for use according to any preceding claim, wherein the MSCs are administered at least once per dosage regime.

13. The isolated population of MSCs for use according to any preceding claim, wherein the MSCs are administered at 3 million cells per kg of subject as 3 separate administrations.

14. The isolated population of MSCs for use according to any preceding claim, wherein the MSCs are administered by intravenous (IV) or intraarterial (IA) administration.

15. The isolated population of MSCs for use according to any preceding claim, wherein the MSCs are not administered directly to the nervous system.

16. The isolated population of MSCs for use according to any preceding claim, wherein the isolated population of MSCs is comprised of at least 50% neural MSCs.

17. The isolated population of MSCs for use according to any preceding claim, wherein the subject is a human subject.

18. The isolated population of MSCs for use according to any preceding claim, wherein the MSCs further comprise at least one drug or agent.

19. A pharmaceutical composition comprising an isolated population of MSCs and a pharmaceutically acceptable excipient, diluent or carrier for use in treating or preventing a disease, disorder or condition of the nervous system in a subject in need thereof; wherein the MSCs express at least one surface marker selected from the group consisting of OSCAR, HAVCR1, ACKR3, C3, SIRPB1, SLC6A6, CCKAR, TNFSF10, CLSTN2, TENM2, SFRP1, PIK3IP1, SCNN1D, CLDN11, ALDH3B1 and ITGB4.

20. A kit comprising an isolated population of MSCs or a pharmaceutical composition comprising an isolated population of MSCs, wherein the MSCs express at least one surface marker selected from the group consisting of OSCAR, HAVCR1, ACKR3, C3, SIRPB1, SLC6A6, CCKAR, TNFSF10, CLSTN2, TENM2, SFRP1, PIK3IP1, SCNN1D,

CLDN11, ALDH3B1 and ITGB4, wherein the kit further comprises instructions for use in treating or preventing a disease, disorder or condition of the nervous system.

21. A method of treating or preventing a disease, disorder or condition of the nervous system in a subject in need thereof, wherein the MSCs express at least one surface marker selected from the group consisting of OSCAR, HAVCR1, ACKR3, C3, SIRPB1, SLC6A6, CCKAR, TNFSF10, CLSTN2, TENM2, SFRP1, PIK3IP1, SCNN1D, CLDN11, ALDH3B1 and ITGB4; and wherein the MSCs are administered at a location such that the MSCs cross the BBB to exert a therapeutic effect to treat or prevent the disease, disorder or condition of the nervous system.

22. A method of making an isolated population of MSCs comprising selecting from TAF MSCs that express at least one surface marker selected from the group consisting of OSCAR, HAVCR1, ACKR3, C3, SIRPB1, SLC6A6, CCKAR, TNFSF10, CLSTN2, TENM2, SFRP1, PIK3IP1, SCNN1D, CLDN11, ALDH3B1 and ITGB4.

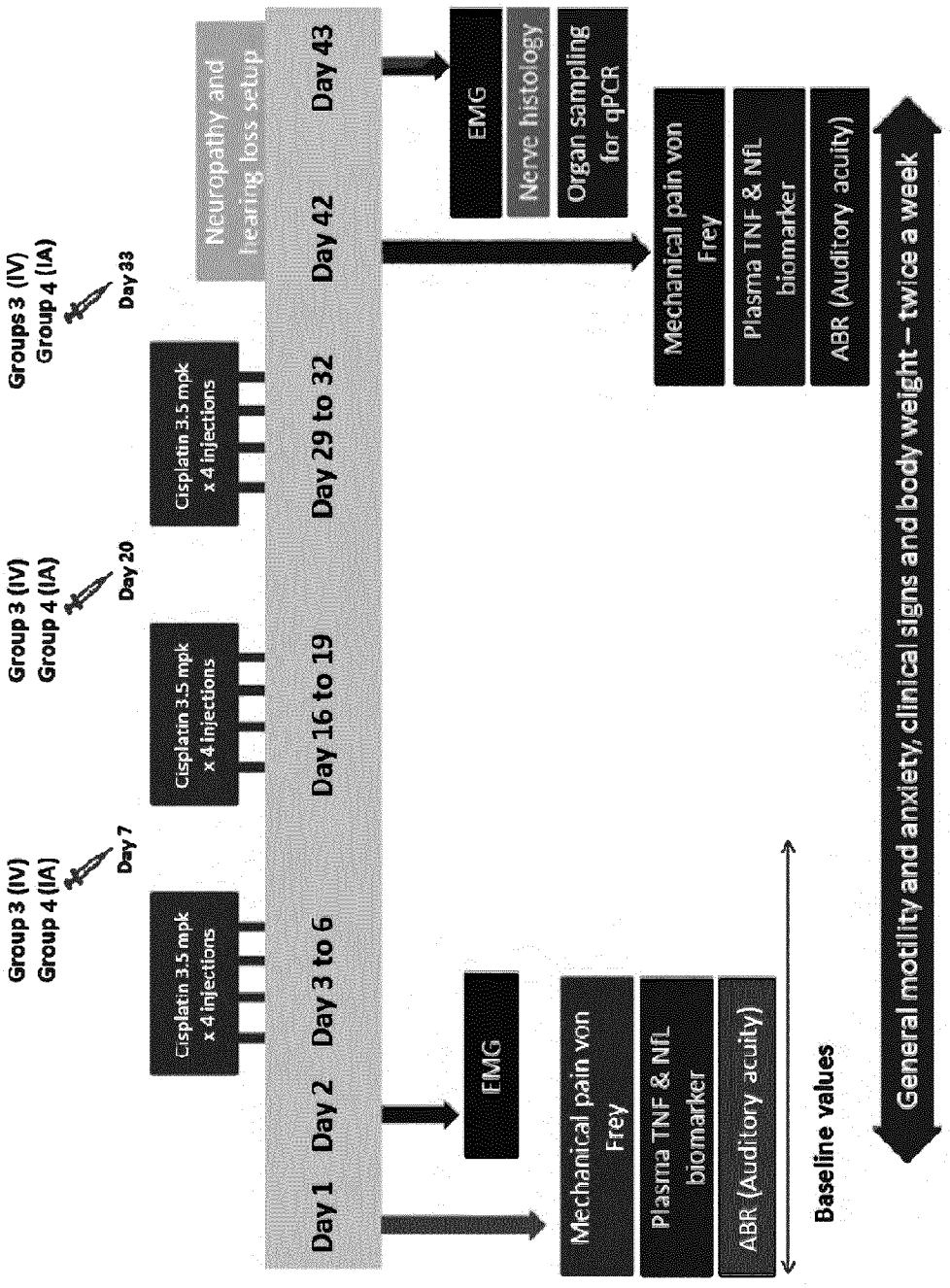
Day 1
Day 2
Day 3 to 6
Day 16 to 19
Day 29 to 32
Day 42
Day 43
Group 3 (IV)
Group 4 (IA)
Day 7
Cisplatin 3.5 mpk x 4 injections
Group 3 (IV)
Group 4 (IA)
Day 20
Cisplatin 3.5 mpk x 4 injections
Groups 3 (IV)
Group 4 (IA)
Day 33
Cisplatin 3.5 mpk x 4 injections
Neuropathy and hearing loss setup
EMG
Mechanical pain von Frey
Plasma TNF & Nfl biomarker
ABR (Auditory acuity)
EMG
Nerve histology
Organ sampling for qPCR
Mechanical pain von Frey
Plasma TNF & Nfl biomarker
ABR (Auditory acuity)
Baseline values
General motility and anxiety, clinical signs and body weight – twice a week

Figure 1

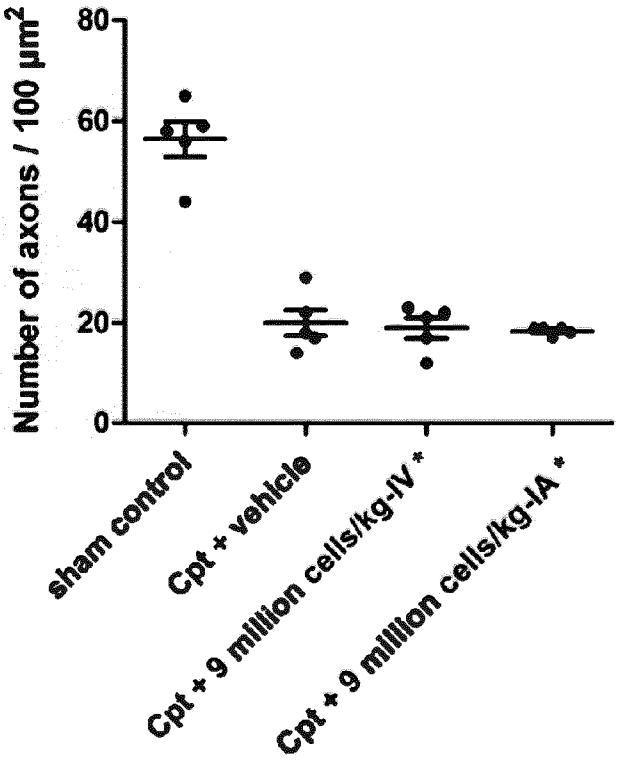
Number of axons / 100 µm²
80
60
40
20
0
sham control
Cpt + vehicle
Cpt + 9 million cells/kg-IV *
Cpt + 9 million cells/kg-IA *

Figure 2

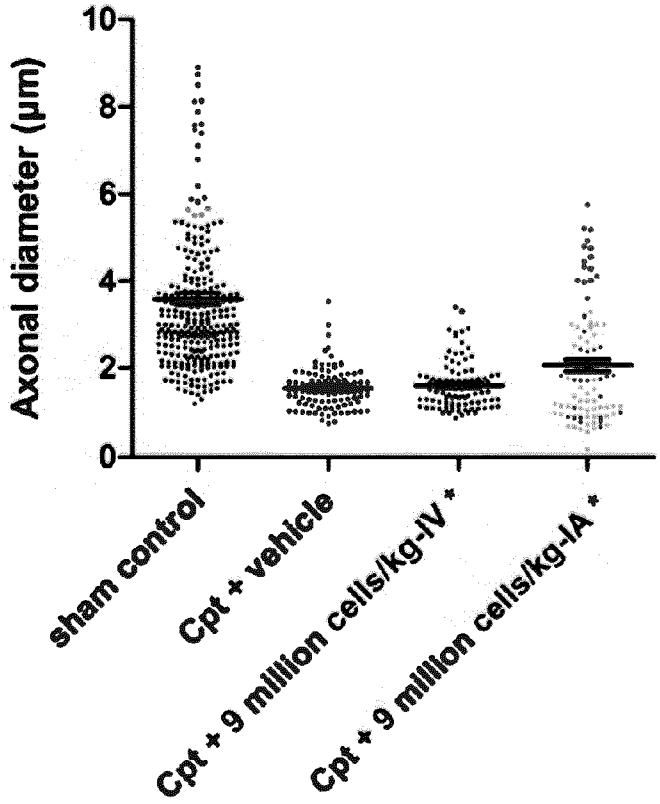
Axonal diameter (µm)
10
8
6
4
2
0
sham control
Cpt + vehicle
Cpt + 9 million cells/kg-IV *
Cpt + 9 million cells/kg-IA *

Figure 3

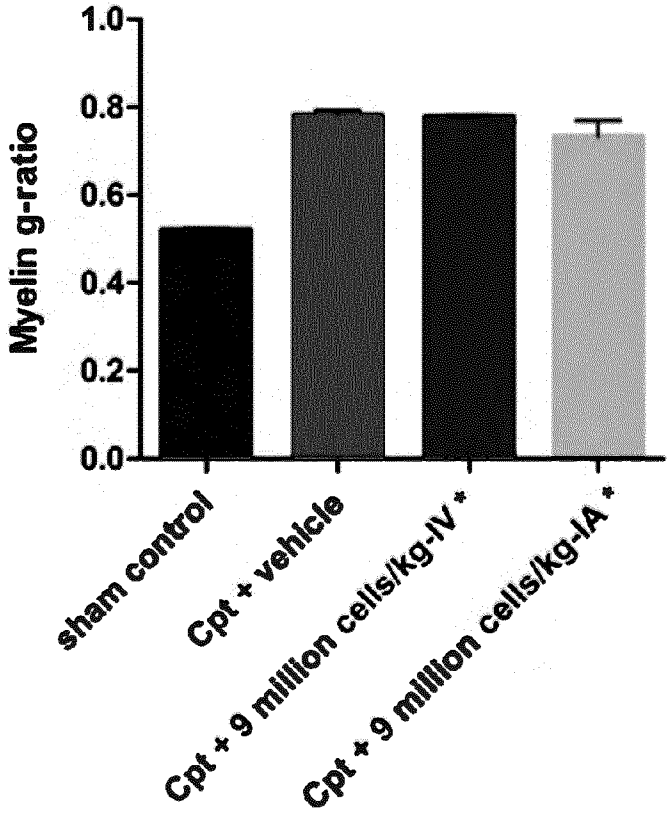
Myelin g-ratio
1.0
0.8
0.6
0.4
0.2
0.0
sham control
Cpt + vehicle
Cpt + 9 million cells/kg-IV *
Cpt + 9 million cells/kg-IA *

Figure 4

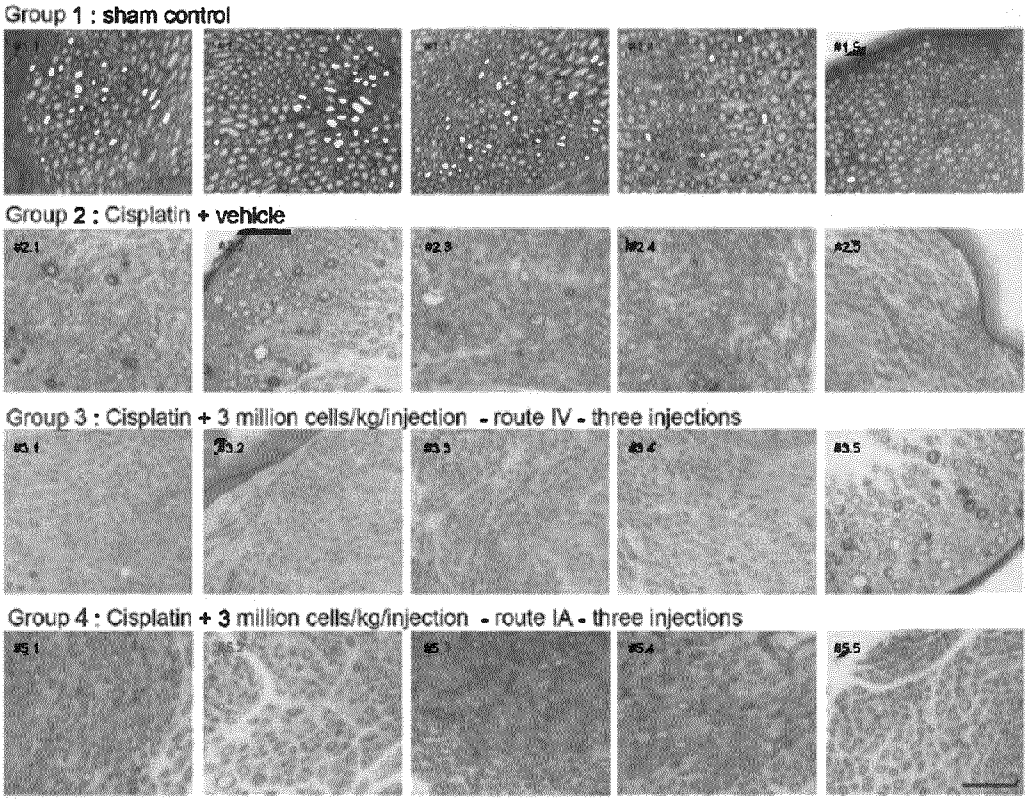
Group 1 : sham control
Group 2 : Cisplatin + vehicle
Group 3 : Cisplatin + 3 million cells/kg/injection - route IV - three injections
Group 4 : Cisplatin + 3 million cells/kg/injection - route IA - three injections

Figure 5

Primers/probe: hAlu Probe ([FAM]TGAGGCAGGAGAATCGCTTGAACC [BHQ1]),

, hAlu forward Primer (TGGTGGCTCTCTCCTGTAAT) and hAlu reverse Primer (GATCTCGGCTCACTGCAAC)

Figure 6

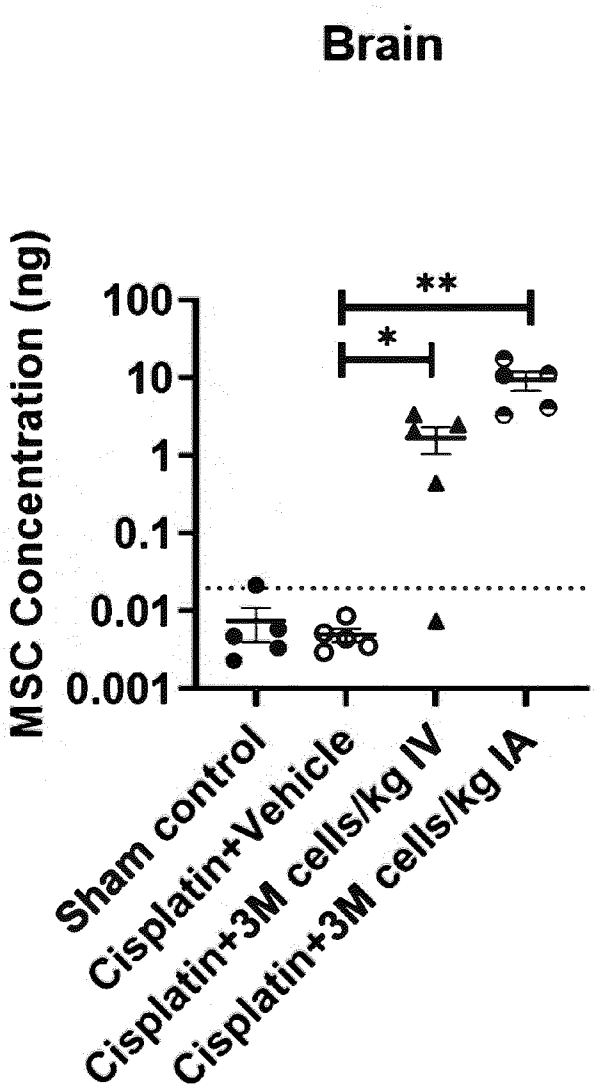
Brain
MSC Concentration (ng)
100
10
1
0.1
0.01
0.001
**
*
Sham control
Cisplatin+Vehicle
Cisplatin+3M cells/kg IV
Cisplatin+3M cells/kg IA

Figure 7

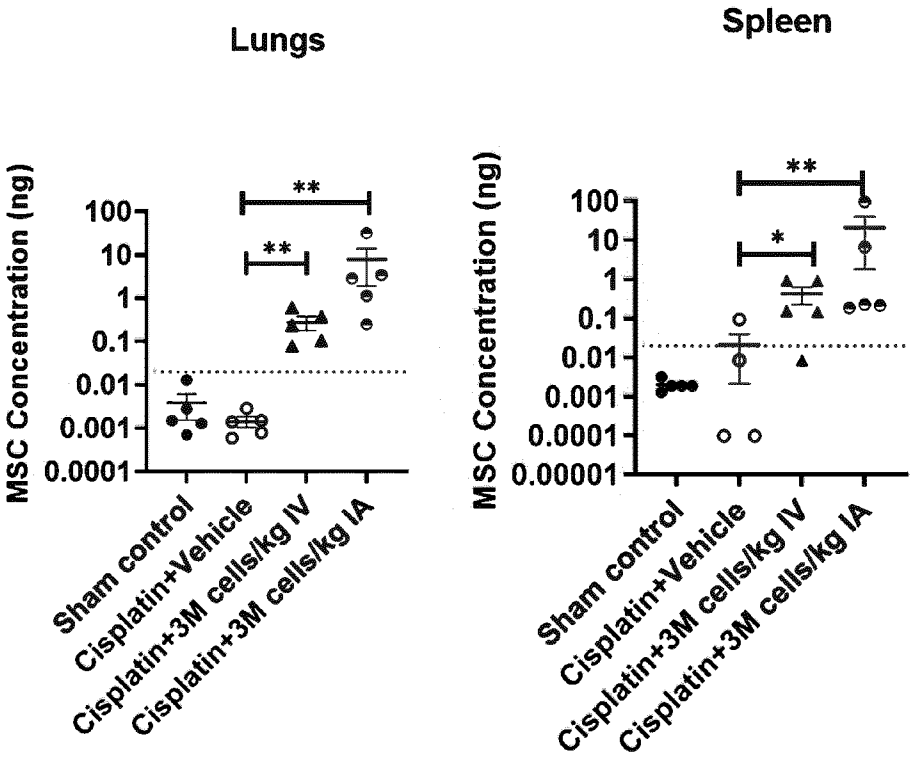
Lungs
Spleen
MSC Concentration (ng)
100
10
1
0.1
0.01
0.001
0.0001
0.00001
**
*
Sham control
Cisplatin+Vehicle
Cisplatin+3M cells/kg IV
Cisplatin+3M cells/kg IA

Figure 8

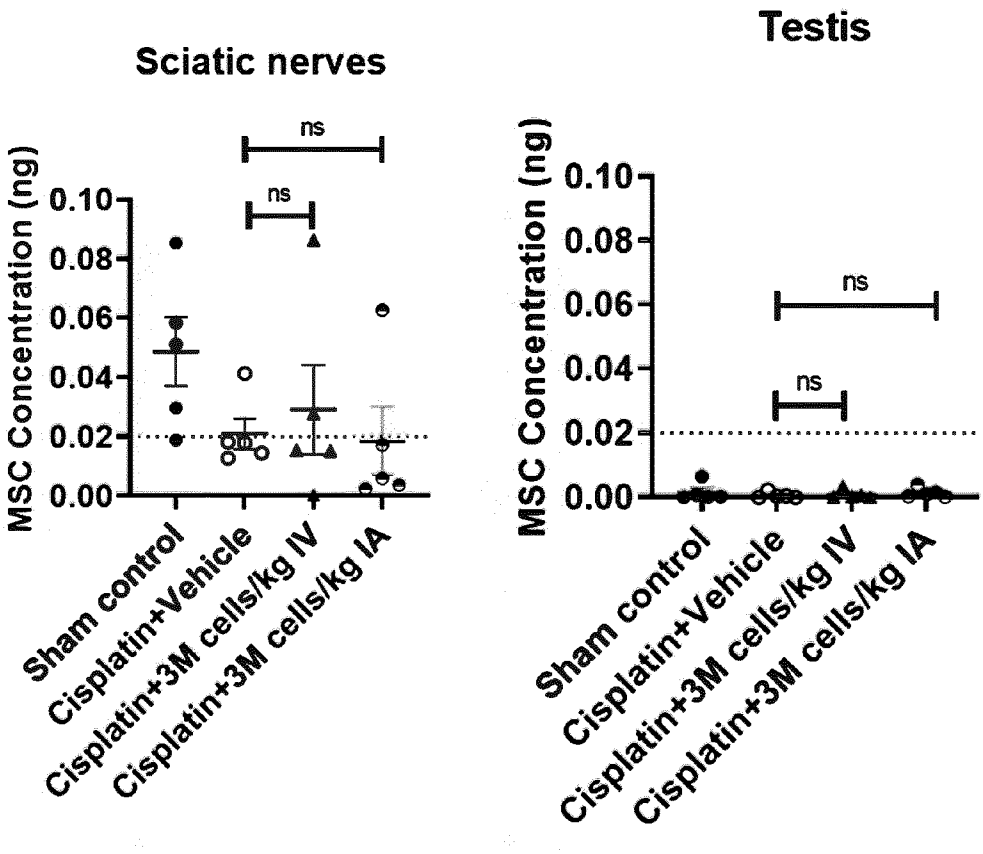
Sciatic nerves
Testis
MSC Concentration (ng)
0.10
0.08
0.06
0.04
0.02
0.00
ns
ns
ns
ns
Sham control
Cisplatin+Vehicle
Cisplatin+3M cells/kg IV
Cisplatin+3M cells/kg IA

Figure 9

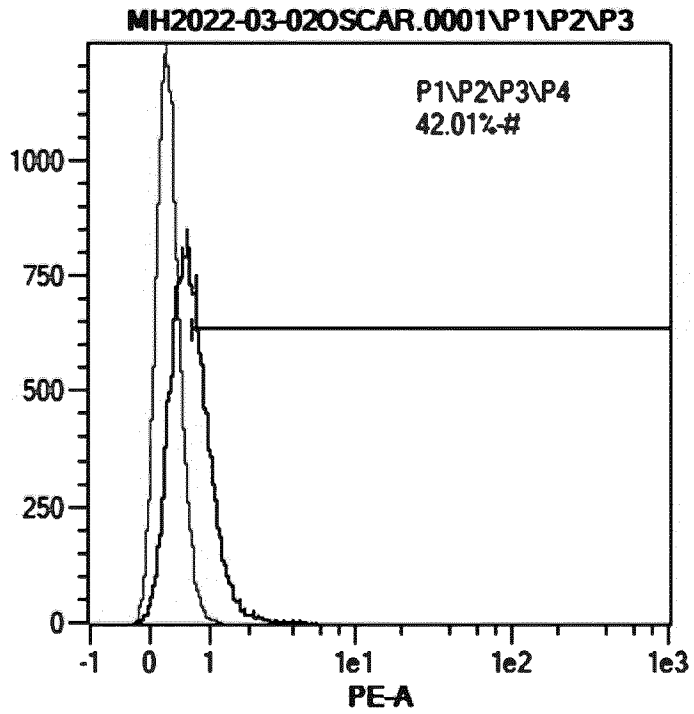
MH2022-03-02OSCAR.0001\P1\P2\P3
P1\P2\P3\P4
42.01%-#
1000
750
500
250
0
-1
0
1
1e1
1e2
1e3
PE-A

Figure 10

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/060787

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K35/28 A61P25/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2020/012991 A1 (ROHTO PHARMA [JP]) 16 January 2020 (2020-01-16) paragraph [0008] - paragraph [0010] -----	1-21
X	WO 2021/076042 A2 (AMNIOTICS AB [SE]) 22 April 2021 (2021-04-22) Pag. 1, 8 -----	1-22
X	WO 2022/195113 A1 (AMNIOTICS AB [SE]) 22 September 2022 (2022-09-22) page 59 - page 60 -----	1-22
X	US 2022/145250 A1 (TALTS JAN [SE] ET AL) 12 May 2022 (2022-05-12) page 30 - page 31 -----	1-22
A	WO 2022/195117 A1 (AMNIOTICS AB [SE]) 22 September 2022 (2022-09-22) the whole document -----	1-22

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 July 2024	02/08/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Dolce, Luca

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2024/060787

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2020012991 A1	16-01-2020	CN 112368370 A	12-02-2021
		JP 2024023760 A	21-02-2024
		JP WO2020012991 A1	15-07-2021
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WO 2021076042 A2	22-04-2021	AU 2020368845 A1	12-05-2022
		CA 3154422 A1	22-04-2021
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