SUMMARY

[0007] Stem cells modified to express therapeutic genes, or other genes of interest, are desirable for a number of different therapeutic and non-therapeutic applications. Traditionally, in the field of prodrug gene therapy, virus-based gene modification approaches have been the favoured approach for modifying stem cells such as MSCs in preclinical and clinical studies, since non-viral approaches have generally provided poor transfection efficiency. However, virus-based gene modification in such applications has inherent safety risk, production of clinical grade virus can be laborious, and the number of gene copies which may be introduced per cell through viral methods is generally low (often < 10 copies per cell). Furthermore, achieving gene modification of stem cells such as MSCs, either virally or non-virally, without causing undesirable changes to phenotype (i.e. multipotency, immunophenotype, tropism, etc.) of the resultant cells is another challenge facing the field.

[0008] As described in detail herein, the present inventors have now developed methods for transfecting mesenchymal stem cells with a nucleic acid construct from which one or more functional genes are expressed, which are non-viral and which in certain examples may provide high transfection efficiency, high copy number per cell, high cell viability, transient expression for extended duration, and/or a substantially unchanged multipotent phenotype. In certain examples, such methods may be scalable and/or suitable for large scale clinical production of modified mesenchymal stem cells. Also described in detail herein are transfected mesenchymal stem cells and populations of mesenchymal stem cells, uses thereof, methods for the treatment of diseases or disorders such as cancer using such transfected stem cells, and kits and compositions relating thereto.

[0009] In one aspect, the present disclosure refers to a mesenchymal stem cell (MSC) transfected with a nucleic acid construct from which one or more functional genes are expressed, the MSC having a multipotent phenotype which is substantially unchanged by the transfection of the nucleic acid construct, and the MSC being free of virus-based transfection vehicle materials.

[0010] In another aspect, the present disclosure refers to a plurality of mesenchymal stem cells (MSCs), wherein at least about 60% of the MSCs are transfected with a nucleic acid construct

from which one or more functional genes are expressed, the transfected MSCs having a multipotent phenotype which is substantially unchanged by the transfection of the nucleic acid construct, and the MSCs being free of virus-based transfection vehicle materials.

[0011] In another example of the plurality of MSCs, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% of the MSCs may be transfected with the nucleic acid construct and express the one or more functional genes. In further examples, a cell viability of the plurality of MSCs may be at least about 70%, at least about 75%, at least about 80%, or at least about 85%.

[0012] In another example, of any of the transfected MSC or MSCs herein, the MSC or MSCs may be each transfected with an average of at least about 1000, at least about 2000, at least about 3000, at least about 4000, at least about 5000, at least about 6000, at least about 7000, at least about 8000, at least about 9000, or at least about 10000 copies of the nucleic acid construct. In another example, the one or more functional genes may be transiently expressed in the transfected MSC cell or cells. In another example, the MSC or MSCs may be derived from cord blood, neonatal birth-associated tissue, Wharton's jelly, umbilical cord, cord lining, placenta, or other source of MSC cells. In another example, the MSC or MSCs may be adipose tissue-derived MSC (AT-MSC), bone marrow-derived MSC (BM-MSC), or umbilical cord-derived MSC (UC-MSC). In another example, the MSCs may be sourced from human, canine, feline, equine, or other species. In another example, the nucleic acid construct may comprise a CpG-free expression plasmid or other CpG-free expression construct, a scaffold/matrix attachment region (S/MAR), an episomal vector, or an EBNA-1 containing construct.

[0013] In another example, of any of the transfected MSC or MSCs herein, the MSC or MSCs may transiently express the one or more functional genes for at least about 7, at least about 8, at least about 9, at least about 10, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, or at least about 17 days following transfection. In another example, the one or more functional genes may comprise a suicide gene. In another example, the one or more functional genes may comprise Cytosine Deaminase (CDy). In another example, the one or more functional genes may comprise

uracil phosphoribosyltransferase (UPRT). In another example, the one or more functional genes may comprise both CDy and UPRT. In another example, the CDy and UPRT may be expressed as a fused construct. In another example, the one or more functional genes may comprise a fluorescent protein. In another example, the fluorescent protein may comprise green fluorescent protein (GFP). In another example, the one or more functional genes may comprise CDy, UPRT, and GFP. In another example, the CDy, UPRT, and GFP may be expressed as a fused construct. In another example, one or more functional genes may comprise herpes simplex virus-1 thymidine kinase (HSV-TK) or another thymidine kinase. In another example, the one or more functional genes may comprise one or more cancer therapy genes, or one or more functional genes which are not related to cancer therapy.

[0014] In another example, of any of the transfected MSC or MSCs herein, the transfected MSC or MSCs may be transfected with the nucleic acid construct using a cationic polymer, a first agent capable of redirecting endocytosed nucleic acids from intracellular acidic compartments, and a second agent capable of stabilizing a microtubular network of the MSC or MSCs. In another example, the cationic polymer may comprise linear or branched polyethylenimine (PEI), poly(amidoamine) PAMAM, or another cationic polymer, or any combination thereof. In another example, the cationic polymer may comprise linear polyethylenimine (LPEI). In another example, the first agent may comprise 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) / cholesteryl hemisuccinate (CHEMS) (DOPE/CHEMS), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) or another fusogenic lipid, or any combinations thereof. In another example, the second agent may comprise a histone deactylase inhibitor (HDACi), such as a histone deactylase 6 inhibitor (HDAC6i). In another example, the second agent may comprise SAHA (Vorinostat).

[0015] In another example or any of the MSC or MSCs herein, the phenotype may include tumor and/or cancer tropism properties of the MSC or MSCs. In another example, the genetically engineered MSC or MSCs of any examples described herein may be sensitive to treatment with 5-fluorocytosine (5FC) or ganciclovir (GCV). One or more examples of the MSC or MSCs may convert: a) 5FC to 5-fluorouracil (5FU), 5-fluorouridine monophosphate (FUMP), or both; b) ganciclovir to ganciclovir monophosphate; or c) a combination of a) and b).

In another example the phenotype may comprise an immunophenotype in which the expression of CD surface markers may be substantially unchanged after transfection.

[0016] In another example of any of the MSC or MSCs described herein, the transfected MSC or MSCs may be plastic-adherent, may express CD105, CD73, and CD90 (>95%), may lack expression of CD45, CD34, CD14, and HLA-DR surface molecules (<2%), and may be capable of differentiating into osteoblasts, adipocytes, and chondroblasts *in vitro*, satisfying the immunophenotype criteria defined by the International Society for Cellular Therapy (ISCT). In another example, the transfected MSC or MSCs may be undifferentiated.

[0017] In another example of any of the MSC or MSCs described herein, the MSC or MSCs may be in a cryopreserved state.

[0018] In another example, the MSC or MSCs may be for use in treating cancer. In certain examples, the cancer may comprise lymphoma, clear cell carcinoma, glioblastoma, temozolomide resistant glioblastoma, perianal carcinoma, oral melanoma, thyroid carcinoma, soft tissue carcinoma, cancer ulceration, nasal tumor, or gastrointestinal cancer, or any combination thereof. In another example, the MSC or MSCs may be for use in combination with 5FC, 5FU, GCV, or any combination thereof.

[0018a] In another aspect, the present disclosure refers to use of the MSC or MSCs as disclosed herein, in the manufacture of a medicament for treating cancer, for example lymphoma, clear cell carcinoma, glioblastoma, temozolomide resistant glioblastoma, perianal carcinoma, oral melanoma, thyroid carcinoma, soft tissue carcinoma, cancer ulceration, nasal tumor, or gastrointestinal cancer.

[0018b] In another aspect, the present disclosure refers to the MSC or MSCs as disclosed herein for use as a medicament.

[0019] In another aspect, the present disclosure refers to a method for transfecting mesenchymal stem cells (MSCs) with a nucleic acid construct from which one or more functional genes are expressed, the method comprising: exposing the MSCs to a transfection mixture comprising the nucleic acid construct which is complexed with a cationic polymer; exposing the MSCs to a first agent capable of redirecting endocytosed nucleic acids from intracellular acidic compartments and a second agent capable of stabilizing a microtubular network of the MSCs; and incubating the MSCs; thereby providing MSCs transfected with the nucleic acid construct.

[0020] In another example of and of the method or methods described herein the MSCs may not be centrifuged during exposure to the transfection mixture, to the first agent and second agent, during incubation, or any combination thereof. In another example the step of incubating the MSCs may comprise gentle mixing without centrifugation. In another example the step of incubating the MSCs may comprise incubating the MSCs for at least

about 2 hours. In another example the step of incubating the MSCs may comprise incubating the MSCs for about 2 hours to about 48 hours. In another example the step of incubating the MSCs may comprise incubating the MSCs for about 3 hours to about 24 hours, or for about 4 hours to about 18 hours.

[0021] In another example of any of the method or methods described herein, the cationic polymer may comprise a cationic polymer which has been identified as having low cytotoxicity against the MSCs. In another example the cationic polymer may have a size of about 5 kDa to about 200 kDa. In another example the cationic polymer may comprise linear or branched polyethylenimine (PEI), poly(amidoamine) PAMAM, or another cationic polymer, or any combinations thereof. In another example the cationic polymer may comprise linear polyethylenimine (LPEI).

[0022] In another example of any of the method or methods described herein, the first agent may comprise 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) / cholesteryl hemisuccinate (CHEMS) (DOPE/CHEMS), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) or another fusogenic lipid, or any combinations thereof. In another example the second agent may comprise a histone deacetylase inhibitor (HDACi), such as a histone deacetylase 6 inhibitor (HDAC6i). In another example the second agent may comprise SAHA (Vorinostat).

[0023] In another example of any of the method or methods described herein, the step of exposing the MSCs to the transfection mixture may comprise complexing the nucleic acid construct with the cationic polymer so as to provide the transfection mixture comprising complexed nucleic acid construct, and adding the transfection mixture to the MSCs. In another example, the step of exposing the MSCs to the transfection mixture may comprise adding the transfection mixture to the MSCs and incubating the MSCs with the transfection mixture. In another example, the step of exposing the MSCs to the first and second agents may comprise replacing the transfection mixture with cell culture media supplemented with the first agent and second agent. In another example, the step of exposing the MSCs to the transfection mixture may comprise removing a culture media from the MSCs and replacing the culture media with the transfection mixture. In another example the step of exposing the MSC to the transfection

mixture may comprise incubating the MSCs with the transfection mixture under mild centrifugation. In another example the mild centrifugation may comprise about 200g for about 5 minutes.

[0024] In another example of any of the method or methods described herein, the cell culture media may comprise complete media. In another example the MSCs may be at about 60% confluency, and the MSCs may be seeded for about 24 hours prior to exposure to the transfection mixture. In another example, the transfection mixture may comprise the complexed nucleic acid construct in serum free DMEM, or in fresh culture media.

[0025] In another example of any of the method or methods described herein, the amount of nucleic acid construct in the transfection mixture to which the MSCs are exposed may be between about 200 to about 500 ng per 1.9 cm^2 surface area. In another example, the amount of nucleic acid construct in the transfection mixture to which the MSCs are exposed may be between about 250 to about 400 ng per 1.9 cm^2 surface area. In another example, the amount of nucleic acid construct in the transfection mixture to which the MSCs are exposed may be between about 300 to about 350 ng per 1.9 cm^2 surface area. In another example, a ratio of cationic polymer to nucleic acid construct may be about 1µg to about 30 µg cationic polymer per 1µg of nucleic acid construct in the transfection mixture. In another example, the transfected MSCs may be each transfected with an average of at least about 1000, at least about 2000, at least about 3000, at least about 4000, at least about 5000, at least about 6000, at least about 7000, at least about 8000, at least about 9000, or at least about 10000 copies of the nucleic acid construct. In another example, the nucleic acid construct may comprise a CpG-free expression plasmid or other CpG-free expression construct, a scaffold/matrix attachment region (S/MAR), an episomal vector, or an EBNA-1 containing construct.

[0026] In another example of any of the method or methods described herein, the one or more functional genes may comprise a suicide gene. In another example, the one or more functional genes may comprise Cytosine Deaminase (CDy) and/or thymidine kinase (TK). In another example, the one or more functional genes may comprise uracil phosphoribosyltransferase (UPRT). In another example, the one or more functional genes may comprise both CDy and UPRT. In another example, the CDy and UPRT may be

expressed as a fused construct. In another example, the one or more functional genes may comprise a fluorescent protein. In another example, the fluorescent protein may comprise green fluorescent protein (GFP). In another example, the one or more functional genes may comprise CDy, UPRT, and GFP. In another example, the CDy, UPRT, and GFP may be expressed as a fused construct. In another example, the one or more functional genes may comprise herpes simplex virus-1 thymidine kinase (HSV-TK) or another thymidine kinase. In another example, the one or more functional genes may comprise one or more cancer therapy genes, or one or more functional genes which are not related to cancer therapy. In another example, the one or more functional genes may be transiently expressed in the transfected MSCs. In another example, the MSCs may transiently express the one or more functional genes for at least about 7, at least about 8, at least about 9, at least about 10, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, or at least about 17 days following transfection. In another example, the one or more functional genes may comprise a fluorescent protein, and the method may further comprise a step of isolating, selecting, or purifying the transfected MSCs using cell sorting or FACS.

[0027] In another example of any of the method or methods described herein, a multipotent phenotype of the transfected MSCs may be substantially unchanged by the transfection. For example, without wishing to be limiting, the multipotent phenotype may include differentiation potential such that the modified cells are able to differentiate to osteogenic, adipogenic and/or chondrogenic lineage, comparable to the native MSCs. In another example, the multipotent phenotype may comprise tumor and/or cancer tropism properties of the MSC. In another example, the multipotent phenotype may comprise an immunophenotype in which the expression of CD surface markers is substantially unchanged after transfection. In another example, the transfected MSCs may be undifferentiated.

[0028] In another example of any of the method or methods described herein, the transfected MSCs may be plastic-adherent, may express CD105, CD73, and CD90 (>95%), may lack expression of CD45, CD34, CD14, and HLA-DR surface molecules (<2%), and may be capable of differentiating into osteoblasts, adipocytes, and chondroblasts *in vitro*, satisfying the immunophenotype criteria defined by the International Society for Cellular Therapy (ISCT).

[0029] In another example of any of the method or methods described herein, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% of the MSCs may be transfected with the nucleic acid construct and express the one or more functional genes. In another example, a cell viability of the transfected MSCs may be at least about 70%, at least about 75%, at least about 80%, or at least about 85%.

[0030] In another example of any of the method or methods described herein, the method may be free of virus-based transfection vehicle materials.

[0031] In another example of any of the method or methods described herein, the MSCs may be derived from cord blood, neonatal birth-associated tissue, Wharton's jelly, umbilical cord, cord lining, placenta, or other source of MSC cells. In another example, the MSCs may be adipose tissue-derived MSC (AT-MSC), bone marrow-derived MSC (BM-MSC), or umbilical cord-derived MSC (UC-MSC). In another example, the MSC or MSCs may be sourced from human, canine, feline, equine, or other species.

[0032] In another example of any of the method or methods described herein, the resultant MSCs may be sensitive to treatment with 5-fluorocytosine (5FC) or ganciclovir (GCV) or both. In another example, the resultant MSC may convert: a) 5FC to 5-fluorouridine (5FU), 5-fluorouridine monophosphate (FUMP) or both; b) ganciclovir to ganciclovir monophosphate; or c) a combination of a) and b).

[0033] In another example of any of the method or methods described herein, the method may comprise a step of culturing the MSCs in a growth medium, such as a fresh growth medium, before the step of exposing the MSCs to the transfection mixture. In another example the step of exposing the MSCs to the transfection mixture may comprise adding the transfection mixture to the MSCs without removing the growth medium from the MSCs, and centrifugation is not performed during the steps of exposing and incubating. In another example, the step of exposing the MSCs to the first agent and the second agent may comprise adding the first and second agent to the MSCs simultaneously, sequentially, or in combination with the transfection mixture. In another example the first and second agent may be added to the MSCs

simultaneously with addition of the transfection mixture to the MSCs, or the first and second agent may be mixed with the transfection mixture and added to the MSCs. In another example the first and second agent may be added to the MSCs shortly after the transfection mixture is added to the MSCs. In another example the transfection mixture may not be removed before the first and second agents are added to the MSCs. In another example a duration of exposure of the MSCs to the transfection mixture may overlap with a duration of exposure of the MSCs to the first and second agents. In another example the transfection mixture may not be removed before the first and second agents are added to the MSCs.

[0034] In another example of any of the method or methods described herein, the method may further comprise a step of cryopreserving the transfected mesenchymal stem cells (MSCs) for storage. In another example, the method may further comprise a step of thawing the cryopreserved transfected mesenchymal stem cells in preparation for use thereof.

[0035] In another example of any of the method or methods described herein, the transfected MSCs are MSCs may be as defined by any of the MSC or MSCs examples described herein. In another example, one or more examples of an MSC, or plurality of MSCs, may be produced by any of the method or methods as described herein.

[0036] In another example, there is provided herein an MSC, or a plurality of MSCs, produced by any of the methods as described herein.

[0037] In another example, there is provided herein a use of any of the MSC or MSCs as defined herein for treating cancer in a subject in need thereof. In certain examples, by way of non-limiting example, the cancer may comprise lymphoma, clear cell carcinoma, glioblastoma, temozolomide resistant glioblastoma, perianal carcinoma, oral melanoma, thyroid carcinoma, soft tissue carcinoma, cancer ulceration, nasal tumor, or gastrointestinal cancer. In another example the MSC or MSCs may be for use in combination with 5FC, 5FU, GCV, or any combination thereof. In another example the MSC or MSCs may be for use in the manufacture of a medicament for the treatment of cancer. In another example the MSC or MSCs may be for use in combination with 5FC, 5FU, GCV, or any combination thereof.

[0038] In another example there is provided herein a method for treating cancer in a subject in need thereof, wherein said method may comprise: administering any of the MSC or MSCs as defined herein to a region in proximity with a cancer cell of the subject, wherein the one or more functional genes in the MSC or MSCs may contribute to an anticancer effect on the cancer cell.

[0039] In certain examples of any of the method or methods for treating cancer described herein, the cancer may comprise lymphoma, clear cell carcinoma, glioblastoma, temozolomide resistant glioblastoma, perianal carcinoma, oral melanoma, thyroid carcinoma, soft tissue carcinoma, cancer ulceration, nasal tumor, or gastrointestinal cancer, for example.

[0040] In another example of any of the method or methods for treating cancer described herein, the MSC or MSCs may be administered simultaneously, sequentially, or in combination with 5FC, 5FU, GCV, or any combination thereof. In another example the one or more functional genes may comprise Cytosine Deaminase (CDy), thymidine kinase (TK), or both. In another example the one or more functional genes may comprise uracil phosphoribosyltransferase (UPRT). In another example the one or more functional genes may comprise both CDy and UPRT. In another example the CDy and UPRT may be expressed in the MSC or MSCs as a fused construct. In another example, the MSC or MSCs may transiently express the one or more functional genes for at least about 7, at least about 8, at least about 9, at least about 10, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, or at least about 17 days following transfection.

[0041] In another example, any of the method or methods for treating cancer described herein may further comprise a step of administering 5FC, 5FU, ganciclovir, or any combination thereof, to the subject such that the MSC or MSCs are exposed to the 5FC, 5FU, ganciclovir or combination thereof.

[0042] In another example, any of the method or methods for treating cancer described herein may further comprise a step of producing the MSC or MSCs according to any of the method or methods as defined in any example described herein prior to the step of administering the MSC or MSCs.

[0043] In another example there is provided herein a composition comprising the engineered MSC or MSCs of any example described herein, and at least one of a pharmaceutically acceptable carrier, diluent, excipient, cell media, or buffer.

[0043a] In another aspect, the present disclosure refers to a pharmaceutical composition comprising the MSC or MSCs as disclosed herein, and at least one of a pharmaceutically acceptable carrier, diluent, excipient, cell media, or buffer.

[0044] In another example there is provided herein a theranostic agent comprising any of the MSC or MSCs of any example described herein.

[0045] In another example there is provided herein a kit for transfecting a mesenchymal stem cell (MSC) with a nucleic acid construct from which one or more functional genes are transiently expressed. In an example the kit may comprise one or more of: an MSC; a nucleic acid construct designed for transient expression of one or more functional genes; a cell culture media; a cationic polymer; a first agent capable of redirecting endocytosed nucleic acids from intracellular acidic compartments; a second agent capable of stabilizing a microtubular network of the MSC; instructions for performing a method as described in any example herein; 5FC; GCV; and/or 5FU. In certain examples, the kit may comprise a cryopreservation buffer or agent, a thawing buffer or agent, or both. In certain non limiting examples, cryopreservation buffers or solutions can be used, such as cryostor10 (Biolife Solutions USA). In further exemplary examples, thawed engineered MSCs can be stored in a hypothermic solution such as Hypothermosol (Biolife Solutions USA). Other examples may also be used as will be apparent to the skilled person in the art.

[0045a] In another aspect, the present disclosure refers to a kit for transfecting a mesenchymal stem cell (MSC) with a nucleic acid construct from which one or more functional genes are transiently expressed, the kit comprising one or more of:

an MSC; a nucleic acid construct designed for transient expression of one or more functional genes; a cell culture media; a cationic polymer;

a first agent capable of redirecting endocytosed nucleic acids from intracellular acidic compartments; a second agent capable of stabilizing a microtubular network of the MSC;

instructions for performing a method as disclosed herein;

5FC;

GCV; and/or

5FU.

[0046] In another example of any of the kit or kits herein, the MSC may be derived from cord blood, neonatal birth-associated tissue, Wharton's jelly, umbilical cord, cord lining, placenta, or other source of MSC cells. In another example the MSC may be an adipose tissue-derived MSC (AT-MSC), bone marrow-derived MSC (BM-MSC), or umbilical cord-derived MSC (UC-MSC). In another example, the MSCs may be sourced from human, canine, feline, equine, or other species. In another example the nucleic acid construct may comprise a CpG-free expression plasmid or other CpG-free expression construct, a scaffold/matrix attachment region (S/MAR), an episomal vector, or an EBNA-1 containing construct. In another example the cationic polymer may comprise linear or branched polyethylenimine (PEI), poly(amidoamine) PAMAM, or another cationic polymer, or any combinations thereof. In another example the cationic polymer may comprise linear

polyethylenimine (LPEI). In another example the first agent may comprise one or more of DOPC, DPPC, or another fusogenic lipid. In another example the first agent may comprise 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) / cholesteryl hemisuccinate (CHEMS) (DOPE/CHEMS), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) or another fusogenic lipid, or any combinations thereof. In another example the second agent may comprise a histone deactylase inhibitor (HDACi) such as a histone deacetylase 6 inhibitor (HDAC6i). In another example the second agent may comprise SAHA (Vorinostat). In another example the one or more functional genes may comprise a suicide gene. In another example the one or more functional genes may comprise Cytosine Deaminase (CDy) or thymidine kinase (TK). In another example the one or more functional genes may comprise uracil phosphoribosyltransferase (UPRT). In another example the one or more functional genes may comprise both CDy and UPRT. In another example, the CDy and UPRT may be expressed as a fused construct. In another example the one or more functional genes may comprise a fluorescent protein. In another example the fluorescent protein may comprise green fluorescent protein (GFP). In another example the one or more functional genes may comprise CDy, UPRT, and GFP. In another example the CDy, UPRT, and GFP may be expressed as a fused construct. In another example the one or more functional genes may comprise herpes simplex virus-1 thymidine kinase (HSV-TK). In another example, the one or more functional genes may comprise one or more cancer therapy genes, or one or more functional genes which are not related to cancer therapy. In another example the cationic polymer may comprise a cationic polymer which has been identified as having low cytotoxicity against the MSCs. In another example the cationic polymer may have a size of about 5 kDa to about 200 kDa. In another example a ratio of cationic polymer to nucleic acid construct in one or more examples of the kit may be about 1µg to about 30 µg cationic polymer per 1µg of nucleic acid construct.

[0047] In another example of any of the kit or kits herein, the kit may be for preparing an MSC-based anti-cancer agent. In another example of any of the kit or kits herein, the kit may comprise instructions and/or apparatus for performing any of the method or methods as defined in any one of the examples described herein.

[0048] In another example there is provided herein a kit for transfecting a mesenchymal stem cell (MSC) with a nucleic acid construct from which one or more functional genes are transiently expressed, wherein the kit may comprise any one or more of: an MSC; a nucleic acid construct designed for transient expression of one or more functional genes; a cell culture media; a cationic polymer; a first agent capable of redirecting endocytosed nucleic acids from intracellular acidic compartments; a second agent capable of stabilizing a microtubular network of the MSC; instructions for performing any of the method or methods described herein; 5FC; GCV; and/or 5FU. In certain examples, the kit may comprise a cryopreservation buffer or agent, a thawing buffer or agent, or both, as described above

[0049] In another aspect, the present disclosure refers to a method for transfecting mesenchymal stem cells (MSCs) with a nucleic acid construct from which one or more functional genes are expressed, the method comprising: culturing the MSCs in a growth medium; adding a transfection mixture comprising the nucleic acid construct which is complexed with a cationic polymer to the MSCs without removing the growth medium from the MSCs; adding a first agent capable of redirecting endocytosed nucleic acids from intracellular acidic compartments and a second agent capable of stabilizing a microtubular network of the MSCs to the MSCs; and incubating the MSCs while in contact with all of the transfection mixture, the first agent, and the second agent for an incubation period; wherein the first and second agents are added to the MSCs simultaneously with the addition of the transfection mixture, sequentially with the addition of the transfection mixture, or in combination with the transfection mixture; and wherein the MSCs are not centrifuged between the adding of the transfection mixture and expiry of the incubation period; thereby providing MSCs transfected with the nucleic acid construct.

[0050] In another example of the method or methods for transfecting mesenchymal stem cells (MSCs) with a nucleic acid construct from which one or more functional genes are expressed as described herein, the incubation period may be at least about 2 hours. In another example, the incubation period may be about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23

hours, about 24 hours, about 25 hours, about 26 hours, about 27 hours, about 28 hours, about 29 hours, about 30 hours, about 31 hours, about 32 hours, about 33 hours, about 34 hours, about 35 hours, or about 36 hours, or more.

[0051] In another example, there is provided herein an MSC cell, or plurality of MSC cells, produced by any of the method or methods for transfecting mesenchymal stem cells (MSCs) with a nucleic acid construct from which one or more functional genes are expressed as described herein. In another example, there is provided herein a use of any of the MSC cell, or plurality of MSC cells, described herein for treating cancer in a subject in need thereof, or for the manufacture of a medicament for the treatment of cancer.

[0052] Methods for treating cancer in a subject in need thereof are described herein. In certain examples of such methods may comprise administering any of the MSC or MSCs as defined in one or more examples described herein to a region in proximity with a cancer cell of the subject, wherein the one or more functional genes in the MSC or MSCs contribute to an anticancer effect on the cancer cell.

[0053] In certain examples, the cancer may comprise lymphoma, clear cell carcinoma, glioblastoma, temozolomide resistant glioblastoma, perianal carcinoma, oral melanoma, thyroid carcinoma, soft tissue carcinoma, cancer ulceration, nasal tumor, or gastrointestinal cancer, for example.

[0054] In another example, there is provided herein a composition comprising any of the MSC or MSCs as described herein, and at least one of a pharmaceutically acceptable carrier, diluent, excipient, cell media, or buffer. In another example, there is provided herein a theranostic agent, and/or a kit, comprising any of the MSC or MSCs of any example described herein.

WHAT IS CLAIMED IS:

1. A mesenchymal stem cell (MSC) transfected with a nucleic acid construct from which one or more functional genes are expressed, the MSC having a multipotent phenotype which is substantially unchanged by the transfection of the nucleic acid construct, and the MSC being free of virus-based transfection vehicle materials.

2. A plurality of mesenchymal stem cells (MSCs), wherein at least about 60% of the MSCs are transfected with a nucleic acid construct from which one or more functional genes are expressed, the transfected MSCs having a multipotent phenotype which is substantially unchanged by the transfection of the nucleic acid construct, and the MSCs being free of virus-based transfection vehicle materials.

3. The MSC or MSCs of claim 1 or 2, wherein the transfected MSC or MSCs are each transfected with an average of at least about 1000, at least about 2000, at least about 3000, at least about 4000, at least about 5000, at least about 6000, at least about 7000, at least about 8000, at least about 9000, or at least about 10000 copies of the nucleic acid construct;

wherein optionally the transfected MSC or MSCs transiently express the one or more functional genes for at least about 7, at least about 8, at least about 9, at least about 10, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, or at least about 17 days following transfection;

wherein optionally the transfected MSC or MSCs are transfected with the nucleic acid construct using a cationic polymer, a first agent capable of redirecting endocytosed nucleic acids from intracellular acidic compartments, and a second agent capable of stabilizing a microtubular network of the MSC or MSCs;

wherein optionally the one or more functional genes comprise a suicide gene;

wherein optionally the one or more functional genes comprise Cytosine Deaminase (CDy), uracil phosphoribosyltransferase (UPRT), or both; and

wherein optionally the multipotent phenotype includes tumor and/or cancer tropism properties of the MSC.

4. The MSC or MSCs of any one of claims 1 - 3, wherein the multipotent phenotype comprises an immunophenotype in which the expression of CD surface markers is substantially unchanged after transfection, preferably wherein the transfected MSC or MSCs are plastic-adherent, express CD105, CD73, and CD90 (>95%), lack expression of CD45, CD34, CD14, and HLA-DR surface molecules (<2%), and are capable of differentiating into osteoblasts, adipocytes, and chondroblasts *in vitro*, satisfying the immunophenotype criteria defined by the International Society for Cellular Therapy (ISCT).

5. Use of the MSC or MSCs of any one of claims 1-4, in the manufacture of a medicament for treating cancer, for example lymphoma, clear cell carcinoma, glioblastoma, temozolomide resistant glioblastoma, perianal carcinoma, oral melanoma, thyroid carcinoma, soft tissue carcinoma, cancer ulceration, nasal tumor, or gastrointestinal cancer.

6. The use according to claim 5, wherein the MSC or MSCs are to be administered in combination with 5FC, 5FU, GCV, or any combination thereof.

7. A method for transfecting mesenchymal stem cells (MSCs) with a nucleic acid construct from which one or more functional genes are expressed, the method comprising:

exposing the MSCs to a transfection mixture comprising the nucleic acid construct which is complexed with a cationic polymer;

exposing the MSCs to a first agent capable of redirecting endocytosed nucleic acids from intracellular acidic compartments and a second agent capable of stabilizing a microtubular network of the MSCs; and

incubating the MSCs;

thereby providing MSCs transfected with the nucleic acid construct.

8. The method of claim 7, wherein the MSCs are not centrifuged during exposure to the transfection mixture, to the first agent and second agent, during incubation, or any combination thereof;

wherein optionally the step of incubating the MSCs comprises incubating the MSCs for about 2 hours to about 48 hours;

wherein optionally the step of exposing the MSCs to the first and second agents comprises replacing the transfection mixture with cell culture media supplemented with the first agent and second agent;

wherein optionally the one or more functional genes comprise a suicide gene;

wherein optionally the one or more functional genes comprise Cytosine Deaminase (CDy), uracil phosphoribosyltransferase (UPRT), or both;

wherein optionally the transfected MSCs are each transfected with an average of at least about 1000, at least about 2000, at least about 3000, at least about 4000, at least about 5000, at least about 6000, at least about 7000, at least about 8000, at least about 9000, or at least about 10000 copies of the nucleic acid construct;

wherein optionally a multipotent phenotype of the transfected MSCs is substantially unchanged by the transfection,

wherein optionally the multipotent phenotype comprises tumor and/or cancer tropism properties of the MSC; and

wherein optionally the multipotent phenotype comprises an immunophenotype in which the expression of CD surface markers is substantially unchanged after transfection, preferably

wherein the transfected MSCs are plastic-adherent, express CD105, CD73, and CD90 (>95%), lack expression of CD45, CD34, CD14, and HLA-DR surface molecules (<2%), and are capable of differentiating into osteoblasts, adipocytes, and chondroblasts *in vitro*, satisfying the immunophenotype criteria defined by the International Society for Cellular Therapy (ISCT).

9. A pharmaceutical composition comprising the MSC or MSCs of any one of claims 1-4, and at least one of a pharmaceutically acceptable carrier, diluent, excipient, cell media, or buffer.

10. A kit for transfecting a mesenchymal stem cell (MSC) with a nucleic acid construct from which one or more functional genes are transiently expressed, the kit comprising one or more of:

an MSC;

a nucleic acid construct designed for transient expression of one or more functional genes;

a cell culture media;

a cationic polymer;

a first agent capable of redirecting endocytosed nucleic acids from intracellular acidic compartments;

a second agent capable of stabilizing a microtubular network of the MSC;

instructions for performing a method as defined in claim 7 or 8;

5FC;

GCV; and/or

5FU.

11. The method of claim 7 or 8, wherein the step of exposing the MSCs to the transfection mixture comprises adding the transfection mixture to the MSCs without removing a growth medium from the MSCs, and centrifugation is not performed during the steps of exposing and incubating.

12. The method of any one of claims 7, 8 or 11, wherein the step of exposing the MSCs to the first agent and the second agent comprises adding the first and second agent to the MSCs simultaneously, sequentially, or in combination with the transfection mixture;

wherein optionally the first and second agent are added to the MSCs simultaneously with addition of the transfection mixture to the MSCs, or wherein the first and second agent are mixed with the transfection mixture and added to the MSCs, or

wherein the first and second agent are added to the MSCs shortly after the transfection mixture is added to the MSCs.

13. The method of claim 12, wherein the transfection mixture is not removed before the first and second agents are added to the MSCs.

14. The method of any one of claims 7, 8 or 11-13, wherein a duration of exposure of the MSCs to the transfection mixture overlaps with a duration of exposure of the MSCs to the first and second agents, preferably wherein the transfection mixture is not removed before the first and second agents are added to the MSCs.

15. A method for transfecting mesenchymal stem cells (MSCs) with a nucleic acid construct from which one or more functional genes are expressed, the method comprising:

culturing the MSCs in a growth medium;

adding a transfection mixture comprising the nucleic acid construct which is complexed with a cationic polymer to the MSCs without removing the growth medium from the MSCs;

adding a first agent capable of redirecting endocytosed nucleic acids from intracellular acidic compartments and a second agent capable of stabilizing a microtubular network of the MSCs to the MSCs; and

incubating the MSCs while in contact with all of the transfection mixture, the first agent, and the second agent for an incubation period;

wherein the first and second agents are added to the MSCs simultaneously with the addition of the transfection mixture, sequentially with the addition of the transfection mixture, or in combination with the transfection mixture; and

wherein the MSCs are not centrifuged between the adding of the transfection mixture and expiry of the incubation period;

thereby providing MSCs transfected with the nucleic acid construct.

16. The MSC or MSCs of any one of claims 1 – 4 for use as a medicament.