could cause variability in clinical cell dosing and complicates the routine application of this technology for the broader PD community. These questions are important for both cell therapy and disease modeling applications.

In human disease modeling, the variation in mDA neuron yield and purity across hPSC lines introduces noise for detecting disease-related phenotypes and complicates drug discovery efforts. Access to defined mDA neuron subtypes would enable studies on the mechanism of cell-type specific vulnerability in PD (Surmeier, *et al*. Cold Spring Harb Perspect Med 2, a009290 (2012); Anderegg, *et al.* FEBS Lett 589, 3714-3726 (2015); Chung, *et al*. Hum Mol Genet 14, 1709-1725 (2005); Brichta and Greengard. Front Neuroanat 8, 152 (2014)). Having access to defined, more robust mDA neuron cultures and the possibility of driving mDA neurons subtypes will greatly accelerate efforts in PD disease modeling and may allow for improved products for mDA neuron cell therapy in the future.

Therefore, there is still a need for improved methods for generating mDA neurons that have improved *in vivo* survival and are suitable for treating neurological disorders such as Parkinson's disease.

3. SUMMARY OF THE INVENTION

The present disclosure provides methods for generating mDA neurons and precursors thereof, mDA neurons and precursors thereof generated by such methods, compositions comprising such cells, and uses of such cells and compositions for preventing and/or treating neurological disorders.

In one aspect, the present disclosure refers to an *in vitro* method for inducing differentiation of stem cells, comprising:

contacting the stem cells with at least one inhibitor of Small Mothers Against Decapentaplegic (SMAD) signaling, at least one activator of Sonic hedgehog (SHH) signaling, and at least one activator of wingless (Wnt) signaling; and

contacting the cells with at least one activator of fibroblast growth factor (FGF) signaling and at least one inhibitor of Wnt signaling to obtain a population of differentiated cells expressing at least one marker indicating a midbrain dopamine neuron or a precursor thereof;

wherein the at least one marker indicating a midbrain dopamine neuron or a precursor thereof is selected from the group consisting of EN1, OTX2, TH, NURR1, FOXA2, PITX3, LMX1A, LMO3, SNCA, ADCAP1, CHRNA4, SOX6, DAT, VMAT2, WNT1, GIRK2, and combinations thereof;

wherein the stem cells are selected from the group consisting of pluripotent stem cells, nonembryonic stem cells, embryonic stem cells, induced pluripotent stem cells, and combinations thereof;

wherein the stem cells are contacted with the at least one inhibitor of Wnt signaling at least 12 days from the initial contact of the stem cells with the at least one inhibitor of SMAD signaling; and

wherein the stem cells are contacted with the at least one activator of FGF signaling at least 12 days from the initial contact of the stem cells with the at least one inhibitor of SMAD signaling.

In yet another aspect, the present disclosure refers to a kit for inducing differentiation of stem cells to midbrain dopamine neurons or precursors thereof, comprising:

(a) at least one inhibitor of SMAD signaling;

(b) at least one activator of SHH signaling;

(c) at least one activator of Wnt signaling;

(d) at least one inhibitor of Wnt signaling;

(e) at least one activator of FGF signaling; and

(f) instructions for inducing differentiation of the stem cells into a population of differentiated cells that express at least one marker indicating a midbrain dopamine neuron or a precursor thereof, wherein the instructions comprise contacting the stem cells with the at least one inhibitor of Wnt signaling at least 12 days from the initial contact of the stem cells with the at least one inhibitor of SMAD signaling and contacting the stem cells with the at least one activator of FGF signaling at least 12 days from the initial contact of the stem cells with the at least one inhibitor of SMAD signaling.

The present disclosure provides *in vitro* methods for inducing differentiation of stem cells. In certain embodiments, the method comprises: contacting the stem cells with at least one inhibitor of Small Mothers Against Decapentaplegic (SMAD) signaling, at least one activator of Sonic hedgehog (SHH) signaling, and at least one activator of wingless (Wnt) signaling; and contacting the cells with at least one activator of fibroblast growth factor (FGF) signaling and at least one inhibitor of Wnt signaling to obtain a population of differentiated cells expressing at least one marker indicating a midbrain dopamine neuron (mDA) or a precursor thereof.

In certain embodiments, the contact of the cells with the at least one inhibitor of Wnt signaling is initiated at least about 5 days from the initial contact of the stem cells with the at least one inhibitor of SMAD signaling. In certain embodiments, the contact of the cells with the at least one inhibitor of Wnt signaling is initiated no later than about 15 days from the initial contact of the stem cells with the at least one inhibitor of SMAD

WHAT IS CLAIMED IS:

1. An *in vitro* method for inducing differentiation of stem cells, comprising:

contacting the stem cells with at least one inhibitor of Small Mothers Against Decapentaplegic (SMAD) signaling, at least one activator of Sonic hedgehog (SHH) signaling, and at least one activator of wingless (Wnt) signaling; and

contacting the cells with at least one activator of fibroblast growth factor (FGF) signaling and at least one inhibitor of Wnt signaling to obtain a population of differentiated cells expressing at least one marker indicating a midbrain dopamine neuron or a precursor thereof;

wherein the at least one marker indicating a midbrain dopamine neuron or a precursor thereof is selected from the group consisting of EN1, OTX2, TH, NURR1, FOXA2, PITX3, LMX1A, LMO3, SNCA, ADCAP1, CHRNA4, SOX6, DAT, VMAT2, WNT1, GIRK2, and combinations thereof;

wherein the stem cells are selected from the group consisting of pluripotent stem cells, nonembryonic stem cells, embryonic stem cells, induced pluripotent stem cells, and combinations thereof;

wherein the stem cells are contacted with the at least one inhibitor of Wnt signaling at least 12 days from the initial contact of the stem cells with the at least one inhibitor of SMAD signaling; and

wherein the stem cells are contacted with the at least one activator of FGF signaling at least 12 days from the initial contact of the stem cells with the at least one inhibitor of SMAD signaling.

2. The method of claim 1, wherein the contact of the cells with the at least one inhibitor of Wnt signaling is initiated 5 days to 15 days from the initial contact of the stem cells with the at least one inhibitor of SMAD signaling; wherein the cells are contacted with the at least one inhibitor of Wnt signaling for 1 day to 30 days.

3. The method of claim 1 or 2, wherein the contact of the cells with the at least one activator of FGF signaling is initiated 5 days to 20 days from the initial contact of the cells with the at least one inhibitor of SMAD signaling; wherein the cells are contacted with the at least one activator of FGF signaling for at least 1 day and/or for up to 20 days; at least 3 days and/or for up to 10 days; or at least 4 days and/or for up to 7 days.

4. The method of any one of claims 1-3, wherein the cells are contacted with the at least one inhibitor of SMAD signaling for 5 days to 7 days.

5. The method of any one of claims 1-4, wherein the cells are contacted with the at least one activator of SHH signaling for 5 days to 7 days.

6. The method of any one of claims 1-5, wherein the cells are contacted with the at least one activator of Wnt signaling for 15 days to 17 days.

7. The method of any one of claims 1-6, wherein the concentration of the at least one activator of Wnt signaling is increased 4 days from its initial contact with the stem cells.

8. The method of claim 7, wherein the concentration of the at least one activator of Wnt signaling is increased by between 200 % and 1000 % from the initial concentration of the at least one activator of Wnt signaling.

9. The method of claim 7 or 8, wherein the concentration of the at least one activator of Wnt signaling is increased to from 1 μM to between 5 μM and 10 μM.

10. The method of any one of claims 1-9, wherein the at least one inhibitor of Wnt signaling is selected from the group consisting of IWP2, IWR1-endo, XAV939, IWP-O1, Wnt-C59, IWP-L6, and ICG-001, and combinations thereof.

11. The method of any one of claims 1-10, wherein the at least one activator of FGF signaling is selected from the group consisting of FGF18, FGF17, FGF8a, FGF8b, FGF4, FGF2, and combination thereof.

12. The method of any one of claims 1-11, wherein the at least one inhibitor of SMAD signaling comprises an inhibitor of TGFβ/Activin-Nodal signaling, an inhibitor of bone morphogenetic protein (BMP) signaling, or a combination thereof; wherein the at least one inhibitor of TGFβ/Activin-Nodal signaling is selected from the group consisting of SB431542, A83-01, and combinations thereof; wherein the at least one inhibitor of BMP signaling is selected from the group consisting of LDN193189, Noggin, dorsomorphin, and combinations thereof.

13. The method of any one of claims 1-12, wherein the at least one activator of Wnt signaling is selected from the group consisting of CHIR99021, CHIR98014, AMBMP hydrochloride, LP 922056, Lithium, deoxycholic acid, BIO, SB-216763, Wnt3A, Wnt1, Wnt5a, and combinations thereof.

14. The method of any one of claims 1-13, wherein the at least one activator of SHH signaling is selected from the group consisting of SHH proteins, recombinant SHHs, modified N-terminal SHHs, SHH C25II, Smoothened agonists (SAG), purmorphamine, and combinations thereof.

15. The method of any one of claims 1-14, wherein at least 80% of the differentiated cells express FOXA2 and EN1 15 days from the initial contact of the stem cells with the at least one inhibitor of SMAD signaling.

16. The method of any one of claims 1-15, further comprising isolating cells that express at least one positive surface marker and do not express at least one negative surface marker; wherein the at least one positive surface marker is selected from the group consisting of CD171, CD184, and combinations thereof; and wherein the at least one negative surface marker is selected from CD49e, CD99, CD340, and combinations thereof.

17. A kit for inducing differentiation of stem cells to midbrain dopamine neurons or precursors thereof, comprising:

(a) at least one inhibitor of SMAD signaling;

(b) at least one activator of SHH signaling;

(c) at least one activator of Wnt signaling;

(d) at least one inhibitor of Wnt signaling;

(e) at least one activator of FGF signaling; and

(f) instructions for inducing differentiation of the stem cells into a population of differentiated cells that express at least one marker indicating a midbrain dopamine neuron or a precursor thereof, wherein the instructions comprise contacting the stem cells with the at least one inhibitor of Wnt signaling at least 12 days from the initial contact of the stem cells with the at least one inhibitor of SMAD signaling and contacting the stem cells with the at least one activator of FGF signaling at least 12 days from the initial contact of the stem cells with the at least one inhibitor of SMAD signaling.