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.

In another aspect, the present disclosure refers to a cell population of *in vitro* differentiated cells, wherein the *in vitro* differentiated cells are obtained by a method as described herein.

In another aspect, the present disclosure refers to a composition comprising the cell population as described herein.

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.

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