

POTENCY ASSAYS FOR VIRAL VECTOR PRODUCTION

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/884,252, filed on August 8, 2019, which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Viral vector mediated gene therapy is a rapidly developing therapeutic field. Many aspects of therapeutic viral vectors will need to be evaluated to determine safety and efficacy prior to use as a therapy.

[0003] Thus, there remains a need for improved methods for determining potency of a recombinant viral vector. In particular, there is a need for methods that allow for improved determination of potency of a payload, such as a polypeptide, expressed by a recombinant viral vector.

SUMMARY

[0004] The present ~~description-disclosure~~ encompasses, *inter alia*, methods for determining potency (e.g., biological activity), e.g., relative potency, of a recombinant viral vector. Methods described herein have improved characteristics over prior methods for determining potency of a payload (e.g., a polypeptide, such as a SMN polypeptide) expressed by a recombinant viral vector. In some ~~embodimentsexamples~~, use of modified host cells with decreased expression of at least one payload (e.g., at least one SMN polypeptide) relative to an unmodified reference host cell of the same type allows for improved potency assays of recombinant viral vectors described herein. In some ~~embodimentsexamples~~, modified host cells (e.g., human modified host cells, e.g., SH-SY5Y KD cells) used in methods described herein are more physiologically relevant and/or easier to culture than other types of host cells (e.g., primary cells and/or non-human mammalian cells, e.g., mouse cells). In some ~~embodimentsexamples~~, SH-SY5Y KD cells comprise a knockdown (e.g., constitutive or conditional) in a *SMN* gene (e.g., a *SMN1* or *SMN2* gene), e.g., comprising or expressing an

inhibitory nucleic acid against a *SMN* gene (e.g., a *SMN1* or *SMN2* gene), e.g., a shRNA against *SMN1* (e.g., a doxycycline inducible shRNA against a *SMN1* gene, e.g., shRNA120 or shRNA 128).

[0005] Such improved characteristics can include, but are not limited to: (i) the method can be performed completely without use of a helper function (e.g., Ad2 or Ad5 helper virus); (ii) lower amounts of recombinant viral vector can be required for transduction than transducing with an unmodified reference host cell of the same type or a different type; (iii) the method has a low standard deviation; (iv) potency can be determined with an accuracy of about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more; (v) potency can be determined with a precision of about 5%, about 6%, about 7%, about 8%, about 9%, or about 10%; (vi) the method can indicate stability of recombinant viral vectors described herein, e.g., following thermal stress of the recombinant viral vector; (vii) potency of recombinant viral vectors described herein is not affected by presence of empty capsids; and/or (viii) able to achieve high signal to noise due to choice of a modified host cell line (e.g., SH-SY5Y KD cells comprising a knockdown (e.g., constitutive or conditional) in a *SMN* gene (e.g., a *SMN1* or *SMN2* gene), e.g., comprising or expressing an inhibitory nucleic acid against a *SMN* gene (e.g., a *SMN1* or *SMN2* gene), e.g., a shRNA against *SMN1* (e.g., a doxycycline inducible shRNA against a *SMN1* gene, e.g., shRNA120 or shRNA 128)).

[0006] In one aspect, the disclosure provides methods of determining potency of a recombinant viral vector encoding at least one *SMN* polypeptide comprising: (a) transducing modified host cells with the recombinant viral vector, wherein the modified host cells comprise decreased expression of at least one *SMN* polypeptide relative to an unmodified reference host cell of the same type; (b) contacting the modified host cells with a first agent for detection of the *SMN* polypeptide; (c) contacting the modified host cells with a second agent comprising a detection moiety for detection of the first agent; and (d) detecting presence of Gemini or coiled bodies (GEMs), thereby determining potency of at least one *SMN* polypeptide. In another aspect, the disclosure provides a method of determining potency of a recombinant viral vector encoding at least one *SMN* polypeptide comprising: (a) transducing modified host cells with the recombinant viral vector, wherein the modified host cells comprise decreased expression of the *SMN* polypeptide relative to an unmodified reference host cell of the same type; (b) contacting

the modified host cells with a first agent for detection of the SMN polypeptide; (c) contacting the modified host cells with a second agent comprising a detection moiety for detection of the first agent; and (d) detecting presence of Gemini of coiled bodies (GEMs), thereby determining potency of the at least one SMN polypeptide.

[0007] In some embodimentsexamples, a recombinant viral vector comprises an adeno-associated viral (AAV) vector, an adenoviral vector, or a retroviral vector. In some embodimentsexamples, a retroviral vector comprises a lentiviral vector or a gammaretroviral vector. In some embodimentsexamples, an AAV vector comprises AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, or a variant thereof. In some embodimentsexamples, an AAV vector comprises AAVhu68. In some embodimentsexamples, an AAV vector comprises a *SMN1* gene operably linked to a chicken- β actin promoter (CB7). In some embodimentsexamples, an AAV vector comprises two ITRs flanking a *SMN1* gene. In some embodimentsexamples, an AAV vector comprises a rabbit β -globin polyA signal.

[0008] In some embodimentsexamples, modified host cells comprise a conditional knockdown or a knockout of an *SMN1* gene. In some embodimentsexamples, modified host cells comprise at least one shRNA for conditional knockdown of an *SMN1* gene. In some embodimentsexamples, at least one shRNA: (i) comprises shRNA120 or shRNA 128, and/or (ii) does not target or affect a recombinant viral vector described herein. In some embodimentsexamples, modified host cells comprise or are mammalian host cells. In some embodimentsexamples, modified host cells comprise or are human cells. In some embodimentsexamples, modified host cells comprise or are SH-SY5Y cells. In some embodimentsexamples, modified host cells comprise or are SH-SY5Y KD cells.

[0009] In some embodimentsexamples, prior to transduction, one or more of the following occurs: (i) host cells are frozen and thawed at least once; (ii) modified host cells are passaged at least 3 times; (iii) host cells are treated with doxycycline (*e.g.*, to induce knockdown of a SMN gene); and/or (iv) host cells are seeded at a density of about 5.0×10^3 to about 5.0×10^4 cells/well.

[0010] In some embodimentsexamples, modified host cells are seeded and transduced within a 24 hour period. In some embodimentsexamples, a transduction step is performed at about 5 different MOIs achieved by serial dilution. In some embodimentsexamples, a transduction step (b) is performed with an MOI of about 6.1×10^5 VG/cell to about 4×10^6

VG/cell (e.g., about 6.1×10^5 VG/cell, about 9.8×10^5 VG/cell, about 1.6×10^6 VG/cell, about 2.5×10^6 VG/cell, and about 4×10^6 VG/cell). In some [embodimentsexamples](#), a signal to noise ratio is greater than or about 2.5.

[0011] In some [embodimentsexamples](#), a first agent comprises an anti-SMN1 antibody or an antigen-binding fragment thereof or an aptamer. In some [embodimentsexamples](#), a detection moiety comprises or is a fluorescent, colorimetric, or enzymatic label. In some [embodimentsexamples](#), a second agent comprises a fluorescently labeled secondary antibody or an antigen-binding fragment thereof. In some [embodimentsexamples](#), presence of GEMs is detected by immunofluorescence. In some [embodimentsexamples](#), presence of GEMs is detected by imaging. In some [embodimentsexamples](#), imaging comprises or is High-Content Imaging (HCI).

[0012] In some [embodimentsexamples](#), methods described herein are performed without or substantially without use of at least one helper function. In some [embodimentsexamples](#), at least one helper function comprises an Ad2 or Ad5 helper virus.

[0013] In some [embodimentsexamples](#), lower amounts of recombinant viral vector are required for transduction than transducing with an unmodified reference host cells of the same type or a different type. In some [embodimentsexamples](#), methods described herein have a low standard deviation.

[0014] In some [embodimentsexamples](#), potency is determined with an accuracy of about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more. In some [embodimentsexamples](#), potency is determined with a precision of about 5%, about 6%, about 7%, about 8%, about 9%, or about 10%.

[0015] In some [embodimentsexamples](#), methods described herein indicate stability of a recombinant viral vector, *e.g.*, following thermal stress of a recombinant viral vector. In some [embodimentsexamples](#), potency of a recombinant viral vector is not affected by presence of empty capsids. In some [embodimentsexamples](#), a recombinant viral vector comprises a plurality of empty virus capsids.

[0016] Any citations to publications, patents, or patent applications herein are incorporated by reference in their entirety. Any numerals used in this application with or without

about/approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art.

[0017] Other features, objects, and advantages of the present invention are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating ~~embodiments~~examples of the present invention, is given by way of illustration only, not limitation. Various changes and modifications within the scope of the invention will become apparent to those skilled in the art from the detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] The Figures described below, which together make up the Drawing, are for illustration purposes only, not for limitation.

CLAIMS

We claim:

1. A method of determining potency of a recombinant viral vector encoding at least one SMN polypeptide comprising:

(a) transducing modified host cells with the recombinant viral vector, wherein the modified host cells comprise decreased expression of the SMN polypeptide relative to an unmodified reference host cell of the same type;

(b) contacting the modified host cells with a first agent for detection of the SMN polypeptide;

(c) contacting the modified host cells with a second agent comprising a detection moiety for detection of the first agent; and

(d) detecting presence of Gemini of coiled bodies (GEMs), thereby determining potency of the at least one SMN polypeptide.

2. The method of claim 1, wherein the recombinant viral vector comprises an adeno-associated viral (AAV) vector, an adenoviral vector, or a retroviral vector.

3. The method of claim 2, wherein the retroviral vector comprises a lentiviral vector or a gammaretroviral vector.

4. The method of claim 2, wherein the AAV vector comprises AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAVhu68, or a variant thereof.

~~5. The method of claim 4, wherein the AAV vector comprises AAVhu68.~~

~~65.~~ The method of claim 2 or 4 ~~or 5~~, wherein the AAV vector comprises one or more of the following:

(a) a SMN1 gene operably linked to a chicken- β actin promoter (CB7)

(b) two ITRs flanking the SMN1 gene; or

(c) a rabbit β -globin polyA signal.

~~7. The method of any one of claims 4-6, wherein the AAV vector comprises two ITRs flanking the *SMN1* gene.~~

~~8. The method of any one of claims 4-7, wherein the AAV vector comprises a rabbit β -globin polyA signal.~~

~~96. The method of any one of claims 1-85, wherein the modified host cells comprise one or both of the following:~~

- ~~(i) a conditional knockdown or a knockout of an *SMN1* gene; or~~
- ~~(ii) at least one shRNA for conditional knockdown of an *SMN1* gene.~~

~~10. The method of any one of claims 1-9, wherein the modified host cells comprise at least one shRNA for conditional knockdown of an *SMN1* gene.~~

~~117. The method of claim 96, wherein the at least one shRNA:~~

- ~~(i) comprises shRNA120 or shRNA 128; and/or~~
- ~~(ii) does not target the recombinant viral vector.~~

~~128. The method of any one of claims 1-117, wherein the modified host cells comprise or are one or more of the following:~~

- ~~(i) mammalian host cells;~~
- ~~(ii) human cells;~~
- ~~(iii) SH-SY5Y cells; or~~
- ~~(iv) SH-SY5Y KD cells.~~

~~13. The method of claim 12, wherein the modified host cells comprise or are human cells.~~

~~14. The method of claim 13, wherein the modified host cells comprise or are SH-SY5Y cells.~~

~~15. The method of claim 14, wherein the modified host cells comprise or are SH-SY5Y KD cells.~~

~~169.~~ The method of any one of claims 1-~~158~~, wherein, prior to transduction, one or more of the following occurs:

- (i) host cells are frozen and thawed at least once;
- (ii) host cells are passaged at least 3 times;
- (iii) host cells are treated with doxycycline; and/or
- (iv) host cells are seeded at a density of about 5.0×10^3 to about 5.0×10^4 cells/well.

~~1710.~~ The method of any one of claims 1-~~159~~, wherein one or more of the following:

- (i) the modified host cells are seeded and transduced within a 24 hour period;
- (ii) the transduction step (b) is performed at about 5 different MOIs achieved by serial dilution;
- (iii) the transduction step (b) is performed with an MOI of about 6.1×10^5 VG/cell to about 4×10^6 VG/cell; or
- (iv) a signal to noise ratio is greater than or about 2.5.

~~18. The method of any one of claims 1-17, wherein the transduction step (b) is performed at about 5 different MOIs achieved by serial dilution.~~

~~19. The method of claim 18, wherein the transduction step (b) is performed with an MOI of about 6.1×10^5 VG/cell to about 4×10^6 VG/cell.~~

~~20. The method of any one of claims 1-19, wherein a signal to noise ratio is greater than or about 2.5.~~

~~211.~~ The method of any one of claims 1-~~2010~~, wherein one or both of:

(a) the first agent comprises an anti-SMN1 antibody or an antigen-binding fragment thereof or an aptamer; or

(b) the detection moiety comprises or is a fluorescent, colorimetric, or enzymatic label.

~~22. The method of any one of claims 1-21, wherein the detection moiety comprises or is a fluorescent, colorimetric, or enzymatic label.~~

2312. The method of claim 2211, wherein the second agent comprises a fluorescently labeled secondary antibody or an antigen-binding fragment thereof.

2413. The method of any one of claims 1-2312, wherein the presence of GEMs is detected by one or both of immunofluorescence or imaging.

~~25. The method of one of claims 1-24, wherein the presence of GEMs is detected by imaging.~~

1426. The method of claim 2513, wherein the imaging comprises or is High-Content Imaging (HCI).

2715. The method of any one of claims 1-2614, wherein the method is performed without or substantially without use of at least one helper function.

2816. The method of claim 2715, wherein the at least one helper function comprises an Ad2 or Ad5 helper virus.

2917. The method of any one of claims 1-2816, wherein lower amounts of recombinant viral vector are required for transduction than transducing with an unmodified reference host cells of the same type or a different type.

3018. The method of any one of claims 1-2917, wherein the method has a low standard deviation.

3419. The method of any one of claims 1-3018, wherein potency is determined with one or both of:

(a) with an accuracy of about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more; or

(b) a precision of about 5%, about 6%, about 7%, about 8%, about 9%, or about 10%.

~~32. The method of claim 31, wherein potency is determined with a precision of about 5%, about 6%, about 7%, about 8%, about 9%, or about 10%.~~

~~33~~20. The method of any one of claims 1-~~32~~19, wherein one or more of the following:

(a) the method indicates stability of the recombinant viral vector, *e.g.*, following thermal stress of the recombinant viral vector

(b) potency of the recombinant viral vector is not affected by presence of empty capsids;
or

(c) the recombinant viral vector comprises a plurality of empty virus capsids.

~~34. The method of any one of claims 1-33, wherein potency of the recombinant viral vector is not affected by presence of empty capsids.~~

~~35. The method of any one of claims 1-34, wherein the recombinant viral vector comprises a plurality of empty virus capsids.~~