

**SYNTHETIC ONCOLYTIC LNP-REPLICON RNA AND
USES FOR CANCER IMMUNOTHERAPY**

RELATED APPLICATION

5 This application claims priority under 35 U.S.C. § 119(e) to United States Provisional Application Serial Number 62/815,611, filed March 8, 2019, the entire contents of which are incorporated by reference herein.

BACKGROUND

10 The present disclosure relates to synthetic oncolytic viruses comprising a lipid nanoparticle comprising one or more types of lipid and a self-amplifying replicon RNA comprising a sequence that encodes an immunomodulatory molecule.

SUMMARY

15 The present disclosure is based, at least in part, on the unexpected discovery that upon injection at a tumor site, a synthetic oncolytic virus successfully triggers anti-cancer immune response of local tumors and enables systemic immunity against distal tumors.

 In one aspect, the present disclosure refers to a synthetic oncolytic virus, comprising:
(i) a lipid nanoparticle comprising one or more types of lipid; and (ii) a self-amplifying
20 replicon RNA comprising a sequence that encodes an interleukin (IL)-12 molecule; wherein the lipid nanoparticle is capable of triggering immunogenic cell death, and wherein the IL-12 molecule is expressed by the self-amplifying replicon RNA.

 In another aspect, the present disclosure refers to a pharmaceutical composition, comprising: the synthetic oncolytic virus as disclosed herein and a pharmaceutically
25 acceptable carrier.

 In another aspect, the present disclosure refers to a use of the synthetic oncolytic virus as disclosed herein or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating cancer in a subject in need thereof.

 Accordingly, one example of the present disclosure provides a synthetic oncolytic
30 virus comprising a lipid nanoparticle comprising one or more types of lipid and a self-amplifying replicon RNA comprising a sequence that encodes an interleukin (IL)-12 molecule. The lipid nanoparticle is capable of triggering immunogenic cell death. The IL-12 molecule is expressed by the self-amplifying replicon RNA.

 The synthetic oncolytic virus comprises a lipid nanoparticle. The lipid nanoparticle
35 comprises one or more types of lipids. In some examples, the one or more types of lipids comprises a cationic lipid. In some examples, the cationic lipid is N1,N3,N5-tris(3-(didodecylamino)propyl)benzene-1,3,5-tricarboxamide (TT3). In some examples, the lipid nanoparticle comprises TT3, 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol, and C14-PEG2000.

40 Further, the synthetic oncolytic virus comprises a self-amplifying replicon RNA. In some examples, the self-amplifying RNA is derived from an alphavirus or other Group IV viruses (positive single strand RNA viruses, such as a hepatitis C virus (HCV)). In some examples, the alphavirus can be Venezuela Equine Encephalitis virus, Semliki Forest virus, or Sindbis virus.

In some examples, the self-amplifying replicon RNA comprises a sequence encoding an IL-12 molecule. In some examples, the sequence that encodes the IL-12 molecule is located in a subgenomic region of the self-amplifying replicon RNA.

5 In some examples, the self-amplifying replicon RNA comprises a nucleotide sequence that is at least 90% identical of the wild type (WT) replicon RNA having a sequence of SEQ ID NO: 1. In some examples, the self-amplifying replicon RNA is not identical to SEQ ID NO: 1 and is capable of expressing the IL-12 molecule at a higher level compared to the self-amplifying replicon RNA comprising SEQ ID NO: 1. In some examples, replicon RNA that is capable of expressing the IL-12 molecule at a higher level comprises a point mutations of
10 G3936C and/or A4758G of SEQ ID NO: 1.

In some examples, the self-amplifying replicon RNA further comprises a serum albumin coding sequence. In some examples, the self-amplifying replicon RNA further comprises a Lumican coding sequence.

15 In some examples, self-amplifying replicon RNA comprises a sequence encoding a IL-12 molecule. In some examples, the IL-12 molecule is selected from the group consisting of IL-12, an IL-12 subunit, or a mutant IL-12 molecule that retains the immunomodulatory function. In some further examples, the IL-12 molecule comprises IL12 α and/or IL12 β subunits.

20 In some examples, the lipid nanoparticle has a diameter of about 100-120 nm. In some examples, the lipid nanoparticle has a zeta potential of about 3-6 mv.

In some examples, the lipid and the self-amplifying replicon RNA have a mass ratio of about 1:2 to 2:1.

The present disclosure, at least in part, relates to a pharmaceutical composition, comprising the synthetic oncolytic virus and a pharmaceutically acceptable carrier.

25 In some examples, pharmaceutical composition is formulated for intratumoral injection.

The present disclosure, at least in part, relates to a method for treating cancer in a subject in need thereof, comprising administering to the subject an effective amount of the synthetic oncolytic virus of any or the synthetic oncolytic virus-containing pharmaceutical
30 composition.

In some examples, the subject is a human patient having or suspected of having a cancer. Exemplary target cancers include, but are not limited to melanoma, breast cancer and colon cancer.

In some examples, the pharmaceutical composition is administered to the subject in a single dose. In some examples, the pharmaceutical composition is administered to the subject by intratumoral injection.

Also within the present disclosure are any of the synthetic oncolytic virus-containing pharmaceutical compositions described herein for use in treating any of the target diseases disclosed herein (e.g., cancer), as well as pharmaceutical compositions comprising the synthetic oncolytic oligonucleotide for use in manufacturing a medicament for cancer treatment.

The details of one or more examples of the invention are set forth in the description below. Other features or advantages of the present invention will be apparent from the following drawings and detailed description of several examples, and also from the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGs. 1A-1H are charts showing the effects of B16F10 cells transfected with DOTAP, lipofectamine (Lipo), TT3 nanoparticle (TT3), mutant replicon RNA (RNA), DOTAP, Lipo, and TT3 nanoparticle encapsulate mutant replicon RNA (DOTAP-mtRep, Lipo-mtRep, and TT3-mtRep), electroporation (Electro), and electroporation with mutant replicon RNA (Electro-mtRep). FIG. 1A is a graph showing B16F10 cell viability 3 days post transfection. FIG. 1B is a graph showing GFP expression in B16F10 cells transfected with lipid nanoparticle encapsulated with mutant or dead replicon RNA (deRep). FIG. 1C is a graph showing percentages of PI+ Annexin V+ dead B16F10 cells transfected with lipid nanoparticles or with lipid nanoparticles encapsulated with replicon RNA. Immunogenic cell death induced by lipid nanoparticles or with lipid nanoparticles encapsulated with replicon RNA, was shown by percent of calreticulin + (CRT+) cells (FIG. 1D), extracellular ATP (FIG. 1E) and HMGB1 release (FIG. 1F). FIG. 1G shows the diameter of the lipid nanoparticles and lipid nanoparticle loaded with mutant replicon RNAs. FIG. 1H shows the zeta potential of the lipid nanoparticles and lipid nanoparticle loaded with mutant replicon RNAs.

FIGs. 2A-2J are charts showing mtRep and deRep trigger TLR3 signaling and induce ISGF3 complex to necrotic cell death. LNP-replicon RNA trigger TLR3 signaling (FIG. 2B and FIG. 2E) and activates the expression of interferon stimulated genes Stat 1, Stat2, IRF9, IRF3 and cGAS (FIGs. 2F-2J), but does not activate TLR2 (FIG. 2A), TLR7 (FIG. 2C) or TLR9 (FIG. 2D).

What is claimed is:

CLAIMS

1. A synthetic oncolytic virus, comprising:
 - (i) a lipid nanoparticle comprising one or more types of lipid; and
 - 5 (ii) a self-amplifying replicon RNA comprising a sequence that encodes an interleukin (IL)-12 molecule;wherein the lipid nanoparticle is capable of triggering immunogenic cell death, and wherein the IL-12 molecule is expressed by the self-amplifying replicon RNA .
- 10 2. The synthetic oncolytic virus of claim 1, wherein the one or more types of lipid comprises a cationic lipid.
3. The synthetic oncolytic virus of claim 2, wherein the cationic lipid is N1,N3,N5-tris(3-(didodecylamino)propyl)benzene-1,3,5-tricarboxamide (TT3).
- 15 4. The synthetic oncolytic virus of claim 2, wherein the lipid nanoparticle comprises TT3, 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol, and C14-PEG2000.
- 20 5. The synthetic oncolytic virus of any one of claims 1-4, wherein the self-amplifying replicon RNA is derived from an alphavirus, or a hepatitis C virus.
6. The synthetic oncolytic virus of any one of claims 5, wherein the alphavirus is Venezuela Equine Encephalitis virus, Semliki Forest virus, or Sindbis virus.
- 25 7. The synthetic oncolytic virus of any one of claims 1-6, wherein the sequence that encodes the IL-12 molecule is located in a subgenomic region of the self-amplifying replicon RNA.
- 30 8. The synthetic oncolytic virus of any one of claim 1-7, wherein the self-amplifying replicon RNA comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 1.

9. The synthetic oncolytic virus of claim 8, wherein the self-amplifying replicon RNA is not identical to SEQ ID NO: 1 and is capable of expressing the IL-12 molecule at a higher level compared to the self-amplifying replicon RNA comprising SEQ ID NO: 1.
- 5 10. The synthetic oncolytic virus of claim 9, wherein the replicon RNA comprises a point mutations of G3936C and/or A4758G of SEQ ID NO: 1.
11. The synthetic oncolytic virus of any one of claims 1-10, wherein the self-amplifying replicon RNA further comprises a serum albumin coding sequence.
- 10 12. The synthetic oncolytic virus of any one of claims 1-11, wherein the self-amplifying replicon RNA further comprises a lumican coding sequence.
13. The synthetic oncolytic virus of any one of claims 1-12, wherein the IL-12 molecule is selected from the group consisting of IL-12, an IL-12 subunit, or a mutant IL-12 molecule that retains the immunomodulatory function.
- 15 14. The synthetic oncolytic virus of claim 13, wherein the IL-12 molecule comprises IL-12 α and/or IL-12 β subunits.
- 20 15. The synthetic oncolytic virus of any one of claims 1-14, wherein the lipid nanoparticle has a diameter of about 100-120 nm.
16. The synthetic oncolytic virus of any one of claims 1-15, wherein the lipid nanoparticle has a zeta potential of about 3-6 mv.
- 25 17. The synthetic oncolytic virus of any one of claims 1-16, wherein the lipid and the self-amplifying replicon RNA have a mass ratio of about 1:1.
- 30 18. A pharmaceutical composition, comprising:
the synthetic oncolytic virus of any one of claims 1-17 and a pharmaceutically acceptable carrier.

19. The pharmaceutical composition of claim 18, wherein the pharmaceutical composition is formulated for intratumoral injection.
20. Use of the synthetic oncolytic virus of any one of claims 1-17 or the pharmaceutical composition of claims 18 or 19 in the manufacture of a medicament for treating cancer in a subject in need thereof.
21. The use of claim 20, wherein the subject is a human patient having or suspected of having a cancer.
22. The use of claim 21, wherein the human patient has a cancer selected from the group consisting of melanoma, breast cancer and colon cancer.
23. The use of any one of claims 19-22, wherein the synthetic oncolytic virus or the pharmaceutical composition is to be administered to the subject in a single dose.
24. The use of any one of claims 19-23, wherein the synthetic oncolytic virus or the pharmaceutical composition is to be administered to the subject by intratumoral injection, intramuscular injection, subcutaneous injection, or intravenous injection.