sequence for human GM-CSF has been inserted into the viral genome at the former ICP34.5 gene sites. *See*, Liu et al., Gene Ther., 10:292-303, 2003.

[0006] Therapeutic combinations of oncolytic viruses and checkpoint inhibitors have been explored. For example, combinations of talimogene laherparepvec and immunotherapies (e.g., ipilimumab and pembrolizumab) are currently being explored in clinical trials in melanoma (NCT01740297 and NCT02263508) and squamous cell carcinoma of the head and neck (NCT02626000).

[0007] Although oncolytic viruses have demonstrated great promise in the treatment of cancer, there remains a need to develop oncolytic viruses that not only limit their replication and lytic damage to cancer cells, but are also able to aid in the mounting and maintenance of a robust systemic anti-tumor immune response.

[0008] The present invention addresses these and other needs.

SUMMARY

[0009] The present disclosure relates to oncolytic viruses comprising a nucleic acid encoding a heterologous dendritic cell growth factor and a nucleic acid encoding a first heterologous cytokine. The heterologous dendritic cell growth factor and first heterologous cytokine may be linked by a polycistronic linker element. In one aspect, the present disclosure relates to an oncolytic virus comprising: a nucleic acid sequence encoding a heterologous dendritic cell growth factor selected from the group consisting of cytokines, C-type lectins, and CD40L; and a nucleic acid sequence encoding a first heterologous cytokine, wherein the cytokine is an interleukin; wherein said nucleic acid sequence encoding a heterologous dendritic cell growth factor and said nucleic acid sequence encoding a first heterologous cytokine are linked by a polycistronic linker element, which is porcine tescho virus 2a (P2A); wherein said oncolytic virus is a herpes simplex virus; and wherein said oncolytic virus further: lacks a functional gene encoding ICP 34.5; and lacks a functional gene encoding ICP 47. In some examples, the polycistronic linker element is porcine tescho virus 2a (P2A) or internal ribosomal entry site (IRES). The oncolytic virus may be a herpes simplex virus, such as a herpes simplex-1 virus. In a particular example, the oncolytic virus is derived from the HSV-1 strain JS1.

[0010] The oncolytic virus may be further modified so that it lacks a functional ICP 34.5 gene and lacks a functional ICP 47 gene.

[0011] In addition, the oncolytic virus may further comprise a promoter wherein the nucleic acid sequences encoding the dendritic cell growth factor and first cytokine are both under the control of the same promoter. In other examples, the oncolytic virus may comprise a first promoter, wherein the nucleic acid sequence encoding the dendritic cell growth factor is under the control of the first promoter; and a second promoter, wherein the nucleic acid sequence encoding the first cytokine is under the control of the second promoter.

[0012] The first heterologous cytokine may be an interleukin, such as interleukin-12 (IL12). The heterologous dendritic cell growth factor may be a second cytokine, such as Fms-related tyrosine kinase 3 ligand (FLT3L).

[0013] In a particular example, the oncolytic virus of the present disclosure comprises an HSV-1 that lacks a functional ICP34.5 encoding gene and lacks a functional ICP47 encoding gene, comprises a

Claims

What is claimed is:

1. An oncolytic virus comprising:

a nucleic acid sequence encoding a heterologous dendritic cell growth factor selected from the group consisting of cytokines, C-type lectins, and CD40L; and

a nucleic acid sequence encoding a first heterologous cytokine, wherein the cytokine is an interleukin;

wherein said nucleic acid sequence encoding a heterologous dendritic cell growth factor and said nucleic acid sequence encoding a first heterologous cytokine are linked by a polycistronic linker element, which is porcine tescho virus 2a (P2A);

wherein said oncolytic virus is a herpes simplex virus; and

wherein said oncolytic virus further:

lacks a functional gene encoding ICP 34.5; and

lacks a functional gene encoding ICP 47.

2. The oncolytic virus according to claim 1, wherein said interleukin is selected from the group consisting of IL2, IL7, IL12, IL15, IL21, and TNF.

3. The oncolytic virus according to claim 1 or 2, wherein said herpes simplex virus is a herpes simplex-1 virus (HSV-1).

4. The oncolytic virus according to claim 3, wherein said oncolytic virus is derived from strain JS1 deposited at the ECAAC under accession number 01010209.

5. The oncolytic virus according to any one of claims 1-4, wherein said oncolytic virus further comprises a promoter, and said nucleic acid sequence encoding the dendritic cell growth factor and said nucleic acid sequence encoding the first cytokine are both under the control of said promoter.

6. The oncolytic virus according to any one of claims 1-5, wherein said interleukin is interleukin-12 (IL12).

7. The oncolytic virus according to any one of claims 1-6, wherein said heterologous dendritic cell growth factor is a second cytokine.

8. The oncolytic virus according to claim 7, wherein said second cytokine is selected from the group comprising Fms-related tyrosine kinase 3 ligand (FLT3L), GMCSF, TNFα, IL36γ, and IFN.

9. The oncolytic virus according to claim 8, wherein said second cytokine is FLT3L.

~~2. The oncolytic virus according to claim 1, wherein said nucleic acid sequence encoding a heterologous dendritic cell growth factor and said nucleic acid sequence encoding a first heterologous cytokine are linked by a nucleic acid sequence encoding a linker element.~~

~~3. The oncolytic virus according to claim 2, wherein said linker element is porcine teschovirus 2a (P2A) or internal ribosomal entry site (IRES).~~

~~4. The oncolytic virus according to any one of claims 1-3, wherein said oncolytic virus is a herpes simplex virus, preferably a herpes simplex-1 virus.~~

~~5. The oncolytic virus according to any one of claims 1-4, wherein said oncolytic virus further:~~

~~lacks a functional gene encoding ICP 34.5; and~~

~~lacks a functional gene encoding ICP 47.~~

~~6. The oncolytic virus according to any one of claims 1-5, wherein said oncolytic virus further comprises a promoter, and said nucleic acid sequence encoding the dendritic cell growth factor and said nucleic acid sequence encoding the first cytokine are both under the control of said promoter.~~

~~7. The oncolytic virus according to any one of claims 1-6, wherein said oncolytic virus further comprises:~~

~~a first promoter, wherein said nucleic acid sequence encoding the dendritic cell growth factor is under the control of said first promoter; and~~

~~a second promoter, wherein and said nucleic acid sequence encoding the first cytokine is under the control of said second promoter.~~

~~8. The oncolytic virus according to any one of claims 1-7, wherein said first heterologous cytokine is an interleukin, preferably wherein said interleukin is interleukin-12 (IL12).~~

~~9. The oncolytic virus according to any one of claims 1-8, wherein said heterologous dendritic cell growth factor is a second cytokine, preferably wherein said second cytokine is Fms-related tyrosine kinase 3 ligand (FLT3L).~~

10. The oncolytic virus according to any one of claims 1-9, wherein said oncolytic virus is a herpes simplex virus 1 (HSV-1) virus,

wherein:

said HSV-1:

lacks a functional gene encoding ICP34.5, and

lacks a functional gene encoding ICP47;

said heterologous dendritic cell growth factor is FLT3L; and

said heterologous first cytokine is IL12.

11. The oncolytic virus according to claim 10, wherein said nucleic acid encoding IL12 and said nucleic acid encoding FLT3L are present in the former site of the gene encoding ICP34.5.

12. The oncolytic virus according to claim 11, ~~wherein said nucleic acid encoding IL12 and said nucleic acid encoding FLT3L are linked via P2A, preferably~~ wherein said nucleic acids encoding IL12, FLT3L, and P2A are present as: [Flt3L]-[P2A]-[IL12]~~, and more preferably wherein said [Flt3L]-[P2A]-[IL12] is under the control of a single promoter~~.

13. The oncolytic virus according to claim ~~13~~12, wherein said [Flt3L]-[P2A]-[IL12] is under the control of a single promoter.

14. The oncolytic virus according to claim 13, wherein said promoter is selected from the list comprising: cytomegalovirus (CMV), rous sarcoma virus (RSV), human elongation factor 1α promoter (EF1α), simian virus 40 early promoter (SV40), phosphoglycerate kinase 1 promoter (PGK), ubiquitin C promoter (UBC), and murine stem cell virus (MSCV).

~~14~~15. The oncolytic virus according to any one of claims ~~1-13~~1-14, wherein said oncolytic virus further comprises a bovine growth hormone polyadenylation signal sequence (BGHpA).

~~15~~16. The oncolytic virus according to any one of claims ~~1-14~~1-15, wherein said oncolytic virus further comprises a nucleic acid that enhances mammalian translation, ~~preferably~~ wherein said nucleic acid that enhances mammalian translation is a Kozak sequence or a consensus Kozak sequence.

17. The oncolytic virus according to claim 16, ~~and more preferably~~ wherein said consensus Kozak sequence is recited in SEQ ID NO: 20.

~~16~~18. The oncolytic virus according to any one of claims ~~1-15~~1-17, wherein said oncolytic virus comprises a nucleic acid, or nucleic acids, encoding [CMV]-[Kozak]-[Flt3L]-[P2A]-[IL12]-[BGHpA].

~~17~~19. The oncolytic virus according to any one of claims ~~1-16~~2-18, wherein said IL12 is present as [P40 subunit]-[GGGGS]-[P35 subunit].

~~18~~20. The oncolytic virus according to claim 19, ~~any one of claims 1-17,~~ wherein the ~~signal peptide in the~~ IL12 P35 subunit ~~is absent~~does not comprise a signal peptide.

~~19. The oncolytic virus according to any one of claims 1-18, wherein said oncolytic virus is derived from strain JS1.~~

~~20~~21. The oncolytic virus according to any one of claims ~~1-19~~1-20, wherein said oncolytic virus comprises:

a FLT3L sequence comprising SEQ ID NO: 1; and

an IL12 sequence comprising SEQ ID NO: 7.

~~21. The oncolytic virus according to claim 20, wherein said oncolytic virus is HSV1/ICP34.5-/ICP47-/FLT3L/IL12.~~

22. The oncolytic virus according to claim 21, wherein said oncolytic virus further comprises:

a CMV promotor comprising SEQ ID NO: 24;

a Kozak sequence comprising SEQ ID NO: 20;

a FLT3L sequence comprising SEQ ID NO: 1;

a P2A sequence SEQ ID NO: 17;

an IL12 sequence comprising SEQ ID NO: 7; and

a BGHpA sequence comprising SEQ ID NO: 21.

23. Use of the oncolytic virus according to any one of claims 1-22 in the manufacture of a medicament for treating cancer.

24. A therapeutically effective amount of the oncolytic virus according to any one of claims 1-22 for use in therapy.

25. The use according to claim 23, wherein said medicament is to be used in combination with a checkpoint inhibitor.

26. A kit comprising an oncolytic virus according to any one of claims 1-22.