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, wherein said oncolytic herpes simplex virus lacks a functional gene encoding ICP 34.5, lacks a functional gene encoding ICP 47, and wherein said oncolytic herpes simplex virus contains a nucleic acid sequence encoding Fms-related tyrosine kinase 3 ligand (FLT3L), and a nucleic acid sequence encoding interleukin-12 (IL12), wherein the nucleic acid sequence encoding FLT3L and the nucleic acid sequence encoding IL12 are linked by a polycistronic linker element porcine tescho virus 2a (P2A), and wherein the nucleic acid sequence encoding FLT3L and the nucleic acid sequence encoding IL12 are both under the control of a single promoter which is cytomegalovirus (CMV) promoter~~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 herpes simplex virus,

wherein said oncolytic herpes simplex virus:

lacks a functional gene encoding ICP 34.5,

lacks a functional gene encoding ICP 47, and

wherein said oncolytic herpes simplex virus contains:

~~comprising:~~

a nucleic acid sequence encoding Fms-related tyrosine kinase 3 ligand (FLT3L), and

a nucleic acid sequence encoding interleukin-12 (IL12);

~~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~~ the nucleic acid sequence encoding FLT3L ~~a heterologous dendritic cell growth factor~~ and ~~said~~ the nucleic acid sequence encoding ~~a first heterologous cytokine~~IL12 are linked by a polycistronic linker element~~, which is~~ porcine tescho virus 2a (P2A); and

wherein the nucleic acid sequence encoding FLT3L and the nucleic acid sequence encoding IL12 are both under the control of a single promoter which is cytomegalovirus (CMV) promoter~~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 herpes simplex virus according to claim 1, wherein said oncolytic herpes simplex virus further comprises a nucleic acid encoding a Kozak sequence~~interleukin is selected from the group consisting of IL2, IL7, IL12, IL15, IL21, and TNF~~.

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

4. The oncolytic herpes simplex virus according to claim 3, wherein said ~~oncolytic virus~~HSV-1 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.~~

~~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~~5. The oncolytic herpes simplex virus according to any one of claims 1-4~~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 acids encoding IL12, FLT3L, and P2A are present as: [Flt3L]-[P2A]-[IL12].~~

~~13. The oncolytic virus according to claim 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).~~

~~15~~6. The oncolytic herpes simplex virus according to any one of claims 1-~~14~~5, wherein said oncolytic herpes simplex virus further comprises a bovine growth hormone polyadenylation signal sequence (BGHpA).

~~16. The oncolytic virus according to any one of claims 1-15, wherein said oncolytic virus further comprises a nucleic acid that enhances mammalian translation, 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, wherein said consensus Kozak sequence is recited in SEQ ID NO: 20.~~

~~18~~7. The oncolytic herpes simplex virus according to any one of claims ~~1~~2-~~17~~6, wherein said nucleic acids encoding CMV, Kozak, Flt3L, P2A, IL12, and BGHpA are present as ~~oncolytic virus comprises a nucleic acid, or nucleic acids, encoding~~ [CMV]-[Kozak]-[Flt3L]-[P2A]-[IL12]-[BGHpA].

~~19~~8. The oncolytic herpes simplex virus according to any one of claims ~~2-18~~1-7, wherein said IL12 is present as [P40 subunit]-[GGGGS]-[P35 subunit].

~~20~~9. The oncolytic herpes simplex virus according to claim ~~19~~8, wherein the ~~IL12 P35 subunit does not comprise a~~ signal peptide in the IL12 P35 subunit is absent.

~~21~~10. The oncolytic herpes simplex virus according to any one of claims 1-~~20~~9, wherein said oncolytic herpes simplex virus comprises:

a FLT3L sequence comprising SEQ ID NO: 1; and

an IL12 sequence comprising SEQ ID NO: 7.

~~22~~11. The oncolytic herpes simplex virus according to any one of claims 2-9~~claim 21~~,

wherein said oncolytic herpes simplex virus is a HSV-1, and

wherein said oncolytic herpes simplex 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.

12. An oncolytic herpes simplex virus, wherein said oncolytic herpes simplex virus is HSV1/ICP34.5-/ICP47-/FLT3L/IL12.

~~23~~13. Use of the oncolytic herpes simplex virus according to any one of claims 1-~~22~~ 12 in the manufacture of a medicament for treating cancer.

~~24~~14. A therapeutically effective amount of the oncolytic herpes simplex virus according to any one of claims 1-~~22~~ 12 for use in therapy.

~~25~~15. The use according to claim ~~23~~13, wherein said medicament is to be used in combination with a checkpoint inhibitor.

16. A pharmaceutical composition comprising an oncolytic herpes simplex virus according to any one of claims 1-12 for use in therapy.

17. The pharmaceutical composition according to claim 16, wherein said composition is to be used in combination with a checkpoint inhibitor.

18~~26~~. A kit comprising an oncolytic herpes simplex virus according to any one of claims 1-~~22~~12.