effects of oncolytic agents and immune co-inhibitory pathway blockade in mice and humans that have so far been observed.

The indoleamine 2,3-dioxygenase (IDO) pathway contributes to tumor-induced tolerance by creating a tolerogenic environment in the tumor and the tumor-draining lymph nodes, both by direct suppression of T cells and enhancement of local regulatory T cell (Treg)-mediated immunosuppression. IDO catalyses the rate-limiting step of tryptophan degradation along the kynurenine pathway, and both the reduction in local tryptophan concentration and the production of immunomodulatory tryptophan metabolites contribute to the immunosuppressive effects of IDO. IDO is chronically activated in many cancer patients with IDO activation correlating with more extensive disease. It can also function as an antagonist to other activators of antitumor immunity. Therefore, inhibitors of the IDO pathway are being developed as anticancer agents, particularly in combination with checkpoint blockade agents such as those which target CTLA-4, PD-1 or PDL-1.

The above discussion demonstrates that there is still much scope for improving oncolytic agents and cancer therapies utilising oncolytic agents.

Summary

The disclosure provides oncolytic viruses expressing a fusogenic protein and at least one immune stimulatory molecule. Oncolytic viruses of the disclosure provide improved treatment of cancer through improved direct oncolytic effects, viral replication and spread through tumors, mediated by the fusogenic protein, which (i) increases the amount of tumor antigens, including neoantigens, which are released for the induction of an anti-tumor immune response; and (ii) enhances the expression of the virus-encoded immune stimulatory molecule(s). Expression of the immune stimulatory molecule(s) further enhances and potentiates the anti-tumor immune effect. Anti-tumor efficacy is improved when an oncolytic virus of the disclosure is used as a single agent and also when the virus is used in combination with other anti-cancer modalities, including chemotherapy, treatment with targeted agents, radiation, immune checkpoint blockade and/or immune potentiating drugs.

Accordingly, in one aspect, the present disclosure provides an oncolytic virus comprising: (i) a fusogenic protein-encoding gene; and (ii) an immune stimulatory molecule-encoding gene. In another aspect, the present disclosure refers to a pharmaceutical composition comprising a virus as described herein and a pharmaceutically acceptable carrier or diluent.

In yet another aspect, the present disclosure refers to the use of the virus as described herein in the manufacture of a medicament for treating cancer. In yet another aspect, the present disclosure refers to the use of the virus as described herein and a further anti-cancer agent in the manufacture of a medicament for combination therapy to treat cancer. In yet another aspect, the present disclosure refers to a product of manufacture comprising a virus as described herein in a sterile vial, ampoule or syringe. The virus may encode more than one fusogenic protein and/or more than one immune stimulatory molecule.

The fusogenic protein is preferably the glycoprotein from gibbon ape leukemia virus (GALV) and has the R transmembrane peptide mutated or removed (GALV-R-). The immune stimulatory molecule is preferably GM-CSF and/or an agonist of an immune co-stimulatory pathway such as GITRL, 4-1-BBL, OX40L, ICOSL or CD40L or a modified version of any thereof. Examples of modified versions include agonists of a co-stimulatory pathway that are secreted rather than being membrane bound, and/or agonists modified such that multimers of the protein are formed. The immune stimulatory molecule may be a protein capable of blocking signaling through CTLA-4, for example an antibody or a fragment thereof which binds CTLA-4.

The virus may be a modified clinical isolate, such as a modified clinical isolate of a virus, wherein the clinical isolate kills two or more tumor cell lines more rapidly and/or at a lower dose *in vitro* than one or more reference clinical isolates of the same species of virus.

The virus is preferably a herpes simplex virus (HSV), such as HSV1. The HSV typically does not express functional ICP34.5 and/or functional ICP47 and/or expresses the US11 gene as an immediate early gene.

The disclosure also provides:

- a pharmaceutical composition comprising a virus of the disclosure and a pharmaceutically acceptable carrier or diluent;
- the virus of the disclosure for use in a method of treating the human or animal body by therapy;
- the virus of the disclosure for use in a method of treating cancer, wherein the method optionally comprises administering a further anti-cancer agent;
- a product of manufacture comprising a virus of the disclosure in a sterile vial, ampoule or syringe;

- a method of treating cancer, which comprises administering a therapeutically effective amount of a virus or a pharmaceutical composition of the disclosure to a patient in need thereof, wherein the method optionally comprises administering a further anti-cancer agent which is optionally an antagonist of an immune co-inhibitory pathway, or an agonist of an immune co-stimulatory pathway;
- use of a virus of the disclosure in the manufacture of a medicament for use in a method of treating cancer, wherein the method optionally comprises administering a further anti-cancer agent which is optionally an antagonist of an immune co-inhibitory pathway, or an agonist of an immune co-stimulatory pathway;
- a method of treating cancer, which comprises administering a therapeutically effective amount of an oncolytic virus, an inhibitor of the indoleamine 2,3-dioxygenase (IDO) pathway and a further antagonist of an immune co-inhibitory pathway, or an agonist of an immune co-stimulatory pathway to a patient in need thereof.

Brief Description of the Figures

Figure 1 depicts the structure of an exemplary virus of the invention that comprises a gene encoding GALV-R- and a gene encoding GM-CSF inserted into the ICP34.5 gene locus, and in which the ICP47 gene is deleted such that the US11 gene is under the control of the ICP47 immediate early promoter. Figure 1 also shows similar viruses expressing only a GALV-R-encoding gene (second panel), or only a GM-CSF-encoding gene (third panel). Also shown is a virus in which the ICP34.5 gene and the ICP47 gene are deleted, but without any inserted genes.

Figure 2 depicts the structure of an exemplary virus of the invention that comprises a gene encoding GALV-R-, a gene encoding GM-CSF and a gene encoding CD40L.

Figure 3 shows the differential abilities of the eight top ranking HSV1 clinical isolate strains as assessed by crystal violet staining 24 hours or 48 hours after infection with a MOI of 0.1, 0.01 or 0.001 as indicated in the Figure to kill Fadu, SK-mel-28, A549, HT1080, MIA-PA-CA-2, HT29 and MDA-MB-231 human tumor cell lines. The virus strains ranked first and second on each cell line are indicated. The virus RH018A was ranked first on each of the Fadu, HT1080, MIA-PA-CA-2 and HT29 cell lines and second on each of the SK-mel-28, A549 and MDA-MB-231 cell lines. RH004A was ranked joint

first with RH018A and RH015A on the HT29 cell line, first on the SK-mel-28 and A549 cell lines and second on the Fadu cell line. RH023A was ranked first on the MDA-MB-231 cell line and second on the HT1080 cell line. RH031A was ranked second on each of the MIA-PA-CA-2 and HT29 cell lines. RH040A was ranked joint second on the HT29 cell line.

CLAIMS

1. An oncolytic virus comprising: (i) a heterologous fusogenic protein-encoding gene; and (ii) an immune stimulatory molecule or an immune stimulatory molecule-encoding gene.

2. The virus of claim 1, wherein the fusogenic protein is selected from the group consisting of vesicular stomatitis virus (VSV) G-protein, syncitin-1, syncitin-2, simian virus 5 (SV5) F-protein, measles virus (MV) H-protein, MV F-protein, respiratory syncytial virus (RSV) F-protein and a glycoprotein from gibbon ape leukemia virus (GALV), murine leukemia virus (MLV), Mason-Pfizer monkey virus (MPMV) and equine infectious anaemia virus (EIAV) from which the R peptide has been deleted.

3. The virus of claim 1, wherein the immune stimulatory molecule is GM-CSF, IL-2, IL-12, IL-15, IL-18, IL-21, IL-24, , a type I interferon, interferon gamma, a type III interferon, TNF alpha, an antagonist of TGF beta, an immune checkpoint antagonist or an agonist of an immune potentiating pathway such as an agonist of CD40, ICOS, GITR, 4-1-BB, OX40 or flt3, optionally CD40 ligand (CD40L), ICOS ligand, GITR ligand, 4-1-BB ligand, OX40 ligand or flt3 ligand.

4. The virus of claim 1, wherein:

(a) the fusogenic protein is the glycoprotein from gibbon ape leukemia virus (GALV) and has the R transmembrane peptide mutated or removed (GALV-R-); and/or

(b) the immune stimulatory molecule is (i) GM-CSF or an agonist of CD40, ICOS, GITR, 4-1-BB, OX40 or flt3, optionally CD40L, GITR ligand, 4-1-BB ligand, OX40 ligand, ICOS ligand or flt3 ligand; or (ii) a CTLA-4 inhibitor.

5. The virus of claim 1, which encodes more than one fusogenic protein and/or more than one immune stimulatory molecule.

6. The virus of claim 5 where the immune stimulatory molecules are GM-CSF and one or more of (i) an agonist of CD40, ICOS, GITR, 4-1-BB, OX40 or flt3, optionally

CD40L, GITR ligand, 4-1-BB ligand, OX40 ligand and ICOS ligand or flt3 ligand; and/or (ii) a CTLA-4 inhibitor.

7. The virus of claim 4, wherein the CTLA-4 inhibitor is a CTLA-4 antibody or a fragment thereof.

8. The virus of claim 1, which is a modified clinical isolate of a virus.

9. The virus of claim 1, which is a modified clinical isolate of a virus, wherein the clinical isolate kills two or more tumor cell lines more rapidly and/or at a lower dose *in vitro* than one or more reference clinical isolates of the same species of virus.

10. The virus of claim 8, wherein the clinical isolate is
strain RH018A having the accession number ECCAC 16121904;
strain RH004A having the accession number ECCAC 16121902;
strain RH031A having the accession number ECCAC 16121907;
strain RH040B having the accession number ECCAC 16121908;
strain RH015A having the accession number ECCAC 16121903;
strain RH021A having the accession number ECCAC 16121905;
strain RH023A having the accession number ECCAC 16121906; or
strain RH047A having the accession number ECCAC 16121909.

11. The virus of claim 1, which is selected from the group consisting of herpes viruses, pox viruses, adenoviruses, retroviruses, rhabdoviruses, paramyxoviruses and reoviruses.

12. The virus of claim 1, which is a herpes simplex virus (HSV).

13. The virus of claim 12 which is a HSV1.

14. The virus of claim 12, wherein the HSV:

(a) does not express functional ICP34.5;

(b) does not express functional ICP47; and/or

(c) expresses the US11 gene as an immediate early gene.

15. The virus of claims 12, wherein a fusogenic protein-encoding gene and an immune stimulatory molecule-encoding gene are inserted into the ICP34.5 encoding locus, either by insertion, or partial or complete deletion, each under separate regulatory control, optionally in a back to back orientation in relation to each other.

16. The virus of claim 1, wherein the sequence of a gene encoding the fusogenic protein and/or the sequence of the gene encoding an immune stimulatory molecule is codon optimized so as to increase expression levels in target cells.

17. A virus according to claim 1, which expresses three heterologous genes, wherein each of the three heterologous genes is driven by a different promoter selected from the CMV promoter, the RSV promoter, the SV40 promoter and a retroviral LTR promoter.

18. The virus of claim 17, which expresses four heterologous genes driven by each of the CMV promoter, the RSV promoter, the SV40 promoter and a retroviral LTR promoter, respectively.

19. The virus of claim 17, where the retroviral LTR is from MMLV.

20. A virus according to claim 1, which expresses three heterologous genes, wherein each of the three heterologous genes is terminated by a different poly adenylation sequence selected from the BGH, SV40, HGH and RBG poly adenylation sequences.

21. The virus of claim 20, which expresses four heterologous genes terminated by each of the BGH, SV40, HGH and RBG poly adenylation sequences, respectively.

22. A pharmaceutical composition comprising a virus according to claim 1 and a pharmaceutically acceptable carrier or diluent.

23. Use of the virus of any one of claims 1 to 21 in the manufacture of a medicament for treating cancer.

24. Use of the virus of any one of claims 1 to 21 and a further anti-cancer agent in the manufacture of a medicament for combination therapy to treat cancer.

25. The use according to claim 24, wherein the further anti-cancer agent is:

(a) selected from an agent targeting an immune co-inhibitory or immune co-stimulatory pathway, radiation and/or chemotherapy, an agent that targets a specific genetic mutation which occurs in tumors, an agent intended to induce an immune response to one or more tumor antigen(s) or neoantigen(s), a cellular product derived from T cells or NK cells, an agent intended to stimulate the STING, cGAS, TLR or other innate immune response and/or inflammatory pathway, a second virus optionally an oncolytic virus, and combinations thereof; or

(b) an antibody.

26. The use according to claim 25, wherein the agent targeting an immune co-inhibitory pathway is a CTLA-4 inhibitor, a PD-1 inhibitor, a PD-L1 inhibitor, a LAG-3 inhibitor, a TIM-3 inhibitor, a VISTA inhibitor, aCSF1R inhibitor, an IDO inhibitor, a CEACAM1 inhibitor, a KIR inhibitor, a SLAMF7 or a CD47 inhibitor and/or the agent targeting an immune co-stimulatory pathway is a GITR agonist, a 4-1-BB agonist, an OX40 agonist, a CD40 agonist or an ICOS agonist.

27. The use according to any one of claims 24 to 26, wherein the virus and the further anti-cancer agent(s) are to be administered separately or concurrently.

28. The use according to any one of claims 24 to 27, wherein the cancer is a solid tumor.

29. A product of manufacture comprising a virus according to claim 1 in a sterile vial, ampoule or syringe.