Summary

In one aspect, the present disclosure refers to an oncolytic virus encoding a CTLA-4 inhibitor. In another aspect, the present disclosure refers to a pharmaceutical composition comprising a virus as disclosed herein and a pharmaceutically acceptable carrier or diluent. In yet another aspect, the present disclosure refers to the virus as disclosed herein for use in therapy. In yet another aspect, the present disclosure refers to the use of the virus as disclosed herein in the manufacture of a medicament for treating cancer. In yet another aspect, the present disclosure refers to the use of the virus a in the manufacture of a medicament for treating tumors that have metastasized from the site of the original tumor.

The present disclosure provides oncolytic viruses expressing an inhibitor of CTLA-4. The virus may further comprise other immunomodulatory agents. In particular the virus may comprise GM-CSF and/or at least one molecule targeting an immune co-stimulatory pathway. The CTLA-4 inhibitor acts to block a co-inhibitory pathway, i.e. interferes with the interaction between CTLA-4 and B7. GM-CSF aids in the induction of an inflammatory tumor micro-environment and stimulates the proliferation and maturation of antigen presenting cells, including dendritic cells, aiding the induction of an anti-tumor immune responses. These immune responses may be amplified through activation of an immune co-stimulatory pathway or pathways using an immune co-stimulatory pathway activating molecule or molecules also delivered by the oncolytic virus.

Oncolytic viruses replicate within tumors, causing lysis of tumor cells and release of tumor antigens, combined with local inflammation and activation of innate immune responses, all of which are beneficial for the activation of an anti-tumor immune response and for the activity of inhibitors of the CTLA-4/B7 interaction.

Delivery of molecules that inhibit the CTLA-4/B7 interaction directly into an immune response initiating-tumor, including where it would be expected to traffic to draining lymph nodes, focuses immune potentiation by the inhibitor on the tumor and therefore on tumor antigens present within it, reduces systemic toxicity and blocks regulatory T cell (Treg) activation that would otherwise inhibit T-cell activation at the site of immune response initiation. The use of an oncolytic virus to deliver molecules targeting CTLA-4, and optionally molecules targeting immune co-stimulatory pathways to tumors focuses the amplification of immune effects on anti-tumor immune responses, and reduces the amplification of immune responses to non-tumor antigens. Thus, immune cells in tumors and tumor draining lymph nodes are selectively affected by the molecules

expressed by the virus rather than immune cells in general. This results in enhanced efficacy of immune cell stimulation, and can also result in reduced off target toxicity. It is also important for focusing the effects of combined systemic immune co-inhibitory pathway blockade and immune co-stimulatory pathway activation on tumors, i.e. such that the amplified immune responses from which co-inhibitory blocks are released are antitumor immune responses rather than responses to non-tumor antigens.

The invention utilizes the fact that, when delivered by an oncolytic virus, the site of action of CTLA-4 blockade and optionally co-stimulatory pathway activation and of GM-CSF expression is in the tumor and/or tumor draining lymph node, but the results of such activation (an amplified systemic anti-tumor-immune response) are systemic. This targets tumors generally, and not only tumors to which the oncolytic virus has delivered the immunomodulatory molecule or molecules. Oncolytic viruses of the invention therefore provide improved treatment of cancer through the generation of improved tumor focused immune responses. The oncolytic virus of the invention also offers improved anti-tumor immune stimulating effects such that the immune-mediated effects on tumors which are not destroyed by oncolysis, including micro-metastatic disease, are enhanced, resulting in more effective destruction of these tumors, and more effective long term anti-tumor vaccination to prevent future relapse and improve overall survival.

Anti-tumor efficacy is improved when an oncolytic virus of the invention 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 and, in preferred examples, immune checkpoint blockade drugs (i.e. antagonists of an immune co-inhibitory pathway, for example antibodies against PD1 or PD-L1) and/or agonists of an immune co-stimulatory pathway.

Accordingly, the present disclosure provides an oncolytic virus encoding a CTLA-4 inhibitor. The CTLA-4 inhibitor is preferably an anti-CTLA-4 antibody or antibody like molecule, or an antigen binding fragment thereof.

The virus may further comprise: (i) a GM-CSF-encoding gene; and/or (ii) an immune co-stimulatory pathway activating molecule or immune co-stimulatory pathway activating molecule-encoding gene. The virus may encode more than one immune co-stimulatory pathway activating molecule/gene.

The immune co-stimulatory pathway activating molecule is preferably 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 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 refers to:

- a pharmaceutical composition comprising a virus of the invention and a pharmaceutically acceptable carrier or diluent;
- the virus of the invention for use in a method of treating the human or animal body by therapy;
- the virus of the invention 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 invention 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 invention to a patient in need thereof, wherein the method optionally comprises administering a further anti-cancer agent;
- use of a virus of the invention 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.

Brief Description of the Figures

Figure 1 depicts the structures of the viruses used to construct exemplary viruses of the invention that comprise anti-mouse or anti-human CTLA-4 constructs that are codon optimized secreted scFv molecules linked to human or mouse IgG1 Fc regions. The scFvs contain light and heavy variable chains from 9D9 (the initial mouse antibody initially used to validate CTLA-4; WO2007/123737: mouse version) or from ipilimumab.

(WO2014/066532; human version) linked by the 15-mer $[G_4S]_3$ (GGGGSGGGGSGGGGS). The viruses are modified versions of strain HSV1 RH018A (clinical strain 18). The ICP34.5 and ICP47 genes are inactivated in the viruses. The US11 gene is placed under the control of the ICP47 immediate early gene promoter by

CLAIMS

1. An oncolytic virus encoding a CTLA-4 inhibitor.

2. The virus of claim 1, wherein the CTLA-4 inhibitor is an anti-CTLA-4 antibody, or an antigen binding fragment thereof.

3. The virus of claim 2, wherein the fragment comprises a scFv molecule, or is a scFv molecule linked to one or more IgG1 constant regions.

4. The virus of claim 2 or 3, wherein the antibody or fragment comprises a light chain variable region sequence linked to an IgG heavy chain.

5. The virus of any one of claims 2 to 4, wherein the antibody or fragment comprises (a) the light chain variable region sequence shown in SEQ ID NO: 1 and the heavy chain variable region sequence shown in SEQ ID NOs: 3; or (b) the light chain variable region sequence shown in SEQ ID NO: 11 and the heavy chain variable region sequence shown in SEQ ID NO: 12; optionally, wherein the antibody or fragment: (a) comprises the amino acid sequence of SEQ ID NO: 9 or 14, and/or (b) is encoded by the nucleotide sequence of SEQ ID NO: 10 or 15.

6. The virus of any one of the preceding claims, wherein the virus further comprises: (a) a GM-CSF-encoding gene; (b) an immune co-stimulatory pathway activating molecule or an immune co-stimulatory pathway activating molecule-encoding gene; and/or (c) a fusogenic protein-encoding gene.

7. The virus of claim 6, wherein the immune co-stimulatory pathway activating molecule-encoding gene encodes: (a) CD40 ligand (CD40L), ICOS ligand, GITR ligand, 4-1-BB ligand, OX40 ligand, TL1A, CD30 ligand, CD27 or flt3 ligand; or (b) CD40 ligand, GITR ligand, 4-1-BB ligand, OX40 ligand, or ICOS ligand.

8. The virus of claim 6 where the fusogenic protein is:

(a) 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) or equine infectious anaemia virus (EIAV) from which the R peptide has been deleted; or

(b) the glycoprotein from gibbon ape leukemia virus (GALV) and has the R transmembrane peptide mutated or removed (GALV-R-).

9. The virus of any one of the preceding claims, which:

(a) encodes more than one immune co-stimulatory pathway activating molecule;

(b) is derived from a clinical isolate of a virus; and/or

(c) 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 any one of the preceding claims, which is:

(a) selected from the group consisting of herpes viruses, pox viruses, adenoviruses, retroviruses, rhabdoviruses, paramyxoviruses and reoviruses;

(b) a herpes simplex virus (HSV);

(c) a HSV1; and/or

(d) a HSV which

(i) does not express functional ICP34.5;

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

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

11. The virus of any one of the preceding claims, which is a HSV or HSV1, wherein an anti-CTLA-4 inhibiting protein encoding gene has been inserted into the ICP34.5 encoding locus by insertion, partial deletion or complete deletion;

optionally wherein the anti-CTLA-4 inhibiting protein encoding gene is included in a cassette also including one or more immune stimulating gene(s) such as GM-CSF and/or an immune co-stimulatory pathway activating molecule encoding gene and/or a fusogenic protein encoding gene.

12. The virus of any one of the preceding claims, wherein the sequence encoding the CTLA-4 inhibitor, the sequence encoding GM-CSF, the sequence encoding an immune co-stimulatory pathway activating molecule and/or the sequence encoding the fusogenic protein is codon optimized so as to increase expression levels in target cells.

13. A virus according to any one of the preceding claims, which expresses:

(a) 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, such as from MMLV, and/or terminated by a different poly adenylation sequence selected from the BGH, SV40, HGH and RBG poly adenylation sequences; or

(b) four heterologous genes driven by each of the CMV promoter, the RSV promoter, the SV40 promoter and a retroviral LTR promoter, such as from MMLV, respectively; and/or terminated by each of the BGH, SV40, HGH and RBG poly adenylation sequences, respectively.

14. A pharmaceutical composition comprising a virus according to any one of claims 1 to 13 and a pharmaceutically acceptable carrier or diluent.

15. The virus of any one of claims 1 to 13 for use in therapy.

16. Use of the virus of any one of claims 1 to 13 in the manufacture of a medicament for treating cancer.

17. Use of the virus of any one of claims 1 to 13 in the manufacture of a medicament for treating tumors that have metastasized from the site of the original tumor.

18. The use according to claim 16 or 17, wherein the method comprises that a further anti-cancer agent is to be administered.

19. The use according to claim 18, wherein the further anti-cancer agent is 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;

optionally wherein the agent targeting an immune co-inhibitory pathway is 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 KIR inhibitor, a SLAMF7 inhibitor, a CEACAM1 inhibitor 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.

20. The use according to claim 18 or 19, wherein:

(a) the further anti-cancer agent is an antibody;

(b) the method comprises that 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 is to be administered;

(c) the virus and the further anti-cancer agent(s) are to be administered separately or concurrently; and/or

(d) the cancer is a solid tumor.