Claims

~~1. A replication competent oncolytic adenovirus with selectivity for cancer cells, wherein the adenovirus comprises a transgene under the control of a promoter endogenous to the virus, wherein the transgene comprises a DNA sequence encoding a B7 protein or an active fragment thereof.~~

~~2. A replication competent oncolytic virus according to claim 1, wherein the B7 protein or active fragment thereof is independently selected from the group comprising B7-1, B7-2, B7-DC, B7-H1, B7-H2, B7-H3, B7-H4, B7-H5 and B7-H6.~~

~~3. A replication competent oncolytic virus according to claim 2, wherein the B7 protein or active fragment thereof is B7-1.~~

~~4. A replication competent oncolytic virus according to claim 1, wherein the virus is a group B adenovirus.~~

~~5. A replication competent oncolytic virus according to any one of claims 1 to 4, wherein the virus is a chimeric virus.~~

~~6. A replication competent oncolytic virus according to claim 5, wherein the virus backbone is enadenotucirev.~~

1. A replication competent oncolytic group B adenovirus enadenotucirev (EnAd) comprising:
two or more transgenes,
wherein a first transgene comprises a DNA sequence encoding B7-1 protein,
wherein an additional transgene encodes an anti-CD3 antibody or binding fragment thereof, and
wherein said transgenes are located between the stop codon and polyA recognition site of the adenoviral gene L5 and the stop codon and polyA recognition site of gene E4, under the control of a major late promoter endogenous to the adenovirus.

~~7~~2. ~~A~~ The replication competent oncolytic virus according to claim ~~6~~1, wherein the virus has a formula (I):

$$5'ITR\text{-}B_1\text{-}B_A\text{-}B_2\text{-}B_X\text{-}B_B\text{-}B_Y\text{-}B_3\text{-}3'ITR \qquad (I)$$

B_1 is a bond or comprises: E1A, E1B or E1A-E1B;

B_A is E2B-L1-L2-L3-E2A-L4;

B_2 is a bond or comprises E3 or a transgene~~, for example under an endogenous or exogenous promoter~~;

B_X is a bond or a DNA sequence comprising: a restriction site, one or more transgenes or both;

B_B comprises L5;

B_Y comprises a transgene cassette comprising a transgene encoding a B7-1 protein ~~or an active fragment thereof~~, a transgene encoding said anti-CD3 antibody or binding fragment thereof, and a regulatory element or a combination of regulatory elements; and

B_3 ~~is a bond or~~ comprises said E4.

3. The replication competent oncolytic virus according to claim 2, wherein the regulatory element is a high efficiency self-cleavable peptide.
4. The replication competent oncolytic virus according to claim 3, wherein the high efficiency self-cleavable peptide is selected from P2A, T2A, F2A and E2A.
5. The replication competent oncolytic virus according to claim 1, wherein one or more proteins encoded by the virus are suitable for expression on the surface of an infected cancer cell, and wherein said proteins are not fusion proteins.
6. The replication competent oncolytic virus according to claim 1, wherein the virus encodes multiple proteins for surface expression on an infected cancer cell.
7. The replication competent oncolytic virus according to claim 6, wherein the proteins are independently processed and expressed in physically separate locations on the cancer cell membrane.
8. ~~A replication competent oncolytic virus according to any one of claims 1 to 7, wherein the B7 protein or active fragment thereof comprises a transmembrane sequence, for example a transmembrane domain from a PDGF receptor, or GPI anchor suitable for anchoring the protein or fragment in a cell membrane.~~
9. ~~A replication competent oncolytic virus according to any one of claims 1 to 8, which further comprises a second transgene, for example encoding a polypeptide selected from the group comprising a cytokine, a chemokine, an antagonistic antibody molecule or fragment thereof, and an agonistic antibody molecule or fragment thereof.~~
10. ~~A replication competent oncolytic virus according to any one of claims 1 to 9, which comprises a second and third transgene, for example encoding two different polypeptides selected from the group comprising a cytokine, a chemokine, an antagonistic antibody molecule or fragment thereof, and an agonistic antibody molecule or fragment thereof.~~
11. ~~A replication competent oncolytic virus according to claim 9 or 10, wherein the second or third transgene encodes a cytokine, selected from the group comprising IL-2, IFN-alpha, IFN-beta, IFN-gamma, Flt3 ligand, GM-CSF, IL-15, and IL-12.~~
12. ~~A replication competent oncolytic virus according to any one of claims 9 to 11, wherein the second or third transgene encodes a chemokine, selected from the group comprising MIP-1 alpha, IL-8, CCL5, CCL17, CCL20, CCL22, CXCL9, CXCL10, CXCL11, CXCL13, CXCL12, CCL2, CCL19 and CCL21.~~
13. ~~A replication competent oncolytic virus according to claim 9 or 12 where a cytokine and a chemokine combination is encoded by the virus selected from the group comprising Mip1α and Flt3 ligand, and Mip1α and IFNα.~~
14. ~~A replication competent oncolytic virus according to any one of claims 1 to 13, wherein the virus encodes an antibody molecule or fragment thereof.~~
15~~8~~. ~~A~~ The replication competent oncolytic virus according to claim ~~14~~1, wherein the antibody or ~~antibody~~ binding fragment ~~molecule~~ further comprises a

transmembrane sequence or GPI anchor such that it is a cell membrane-anchored form ~~or a transmembrane domain, for example from a PDGF receptor~~ .

9. The replication competent oncolytic virus according to claim 1, wherein the anti-CD3 is a scFv antibody fragment.

10. The replication competent oncolytic virus according to claim 1, wherein the B7-1 protein is a full-length protein.
11. The replication competent oncolytic virus according to claim 1, wherein the B7-1 protein comprises a transmembrane domain or GPI anchor.
12. The replication competent oncolytic virus according to claim 1, wherein the B7-1 protein comprises a transmembrane domain from a B7 protein.
13. The replication competent oncolytic virus according to claim 1, wherein the virus comprises a third transgene encoding a polypeptide selected from the group comprising a cytokine, a chemokine, a ligand, an antagonistic antibody molecule, and an agonistic antibody molecule.
14. The replication competent oncolytic virus according to claim 1, wherein the B7-1 protein is human.
15. The replication competent oncolytic virus according to claim 14, wherein the B7-1 protein has the sequence shown in SEQ ID NO: 11 or a sequence at least 95% identical thereto.
16. ~~A~~ The replication competent oncolytic virus according to claim ~~14 or 15~~1, wherein the antibody ~~or antibody fragment~~ molecule or binding fragment thereof comprises an anti-human CD3 antigen binding domain.
17. The replication competent oncolytic virus according to claim 16, wherein the binding fragment has a sequence shown in SEQ ID NOs: 15 or 99.
18. The replication competent oncolytic virus according to claim 1, comprising SEQ ID NO: 96.
~~17. A replication competent oncolytic virus according to claim 14 to 16, wherein the antibody molecule is an inhibitor, for example selected from the group comprising an inhibitor of an angiogenesis factor, such as an anti-VEGF antibody molecule, and inhibitor of T cell deactivation factors, such an anti-CTLA-4 antibody molecule.~~
~~18. A replication competent oncolytic virus according to claim 14 to 17, wherein the antibody molecule is an agonist, for example of one or more selected from the group comprising CD40, GITR, OX40, CD27 and 4-1BB.~~
19. A pharmaceutical composition comprising ~~an replication comprising a~~ the replication competent oncolytic ~~adeno~~virus according to ~~any one of~~ claim~~s~~ 1~~ to 18~~, and a pharmaceutically acceptable excipient~~diluent or carrier~~.
~~20. A method of treating a patient comprising administering a therapeutically effective amount of a replication competent adenovirus according to any one of claims 1 to 18 or a composition according to claim 19.~~
~~21. A replication competent adenovirus according to claim 1 to 18 or a composition according to claim 18 for use in treatment.~~
~~22. Use of a replication competent adenovirus according to claim 1 to 18 or a composition according to claim 18 for use in the manufacture of a medicament, for example for the treatment of cancer.~~
~~23. A method of generating an replication competent adenovirus according to claim 1 to 18 by replication in a host cell.~~

20. The replication competent oncolytic virus according to claim 1, wherein E4 open reading frame 4 is deleted.