

The present disclosure also extends to any virus sequence or cassette sequence specifically disclosed herein, compositions comprising said viruses, use of the said cassettes to generate viruses, and use of viruses according to the disclosure in therapy, in particular cancer therapy.

5 The present application claims priority for GB1708779.2 and GB1708778.4 both filed 1 June 2017. The disclosure of each is incorporated herein by reference, in particular incorporated are the amino acid and polynucleotide sequences disclosed therein. The priority documents may be employed as basis for a correction to the present specification.

10 The present invention is further described by way of illustration only in the following examples.

EXAMPLES

Example 1: Production of EnAd viruses expressing anti-CD40 monoclonal antibodies (NG-350).

15 The first enadenotucirev virus genome generated encoding an anti-CD40 monoclonal antibody was designated NG-350 (SEQ ID NO. 13).

To produce the NG-350 genome a plasmid, pNG-350, was generated by direct insertion of a cassette encoding; a 5' short splice acceptor sequence (CAGG, SEQ ID NO.2); a heavy chain leader sequence (SEQ ID NO. 3), the anti-CD40 VH chain (SEQ ID NO. 4), antibody heavy chain constant region (SEQ ID NO. 5), a P2A high efficiency self-cleavable peptide (SEQ ID
20 NO. 6), a light chain leader sequence (SEQ ID NO. 7), the anti-CD40 VL chain (SEQ ID NO. 8), an antibody light chain constant region (SEQ ID NO. 9) and a SV40 poly(A) tail (SEQ ID NO.10), into the plasmid pEnAd2.4. A schematic of the transgene cassette (SEQ ID NO. 11) is shown in Figure 1.

Virus Production and characterisation

25 The plasmid pNG-350 was linearised by restriction digest with the enzyme *AscI* to produce the virus genome NG-350 (SEQ ID NO. 13). The virus NG-350 was amplified and purified according to methods given below.

Digested DNA was purified by phenol/chloroform extraction and precipitated for 16hrs, -
30 20°C in 300µl >95% molecular biology grade ethanol and 10µl 3M Sodium Acetate. The precipitated DNA was pelleted by centrifuging at 14000rpm, 5 mins and was washed in 500µl 70% ethanol, before centrifuging again, 14000rpm, 5mins. The clean DNA pellet was air dried, resuspended in 500µl OptiMEM containing 15µl lipofectamine transfection reagent and incubated for 30 mins, RT. The transfection mixture was then added drop wise
35 to a T-25 flask containing 293 cells grown to 70% confluency. After incubation of the cells with the transfection mix for 2hrs at 37°C, 5% CO₂ 4mls of cell media (DMEM high glucose with glutamine supplemented with 2% FBS) was added to the cells and the flasks was incubated 37°C, 5% CO₂.

40 The transfected 293 cells were monitored every 24hrs and were supplemented with additional media every 48-72hrs. The production of virus was monitored by observation of

CLAIMS

1. A replication competent oncolytic group B adenovirus comprising a transgene cassette encoding an anti-CD40 antibody or antigen binding fragment thereof, wherein the transgene cassette comprises a nucleic acid sequence given in SEQ ID NO: 12.
2. An oncolytic virus according to claim 1, wherein the virus is Enadenotucirev.
3. An oncolytic virus according to any one of claims 1 to 2, wherein the virus comprises SEQ ID NO: 1.
4. An oncolytic virus according to claim 3, which consists of SEQ ID NO: 1.
5. A pharmaceutical composition comprising a virus according to any one of claims 1 to 4, and a pharmaceutically acceptable excipient, diluent or carrier.
6. An oncolytic virus according to any one of claims 1 to 4 or a pharmaceutical composition according to claim 5, for use in treatment.
7. Use of an oncolytic virus according to any one of claims 1 to 4 or a pharmaceutical composition according to claim 5 in the manufacture of a medicament for the treatment of cancer, insulin resistance, obesity and/or immune deficiency.
8. The use according to claim 7, wherein the virus or composition is for use in the treatment of cancer.
9. A combination therapy (for example for use in the treatment of cancer) comprising a virus according to any one of claims 1 to 4 or a composition according to claim 5 and a further anti-cancer therapy.
10. A combination therapy according to claim 9, wherein the further anti-cancer therapy is chemotherapy.
11. A combination therapy according to claim 9 or 10, wherein the further anti-cancer therapy is a check point inhibitor.

12. A combination therapy according to claim 11, wherein the anti-cancer therapy is selected from the group comprising a PD-1 inhibitor, a PD-L1 inhibitor, a CTLA-4 inhibitor, a TIM-3 inhibitor, a LAG-3 inhibitor, a TIGIT inhibitor, a B7-H3 (CD276) inhibitor, a B7-H4 (B7S1) inhibitor, a B7H7 (HHLA2) inhibitor, a CD96 inhibitor, a VISTA inhibitor and a combination of two or more of the same.
13. A combination therapy according to claim 12, wherein the inhibitor is an antibody or antigen binding fragment thereof.
14. A combination therapy according to any one of claims 9 to 11, wherein the further anti-cancer therapy is a costimulatory pathway agonist.
15. A combination therapy according to claim 14, wherein the further anti-cancer therapy is selected from the group comprising a CD27 agonist, a CD28 agonist, an ICOS agonist, a TMIGD2 (IGPR-1/CD28H) agonist, a CD226 agonist, an OX40 agonist, a 4-1BB agonist, and a combination of two or more of the same.
16. A combination therapy according to claim 15, wherein the further anti-cancer therapy is an antibody or antigen binding fragment thereof.
17. A combination therapy according to any one of claims 9 to 16, wherein the further anti-cancer therapy activates immune responses or reverses suppression of immune responses.
18. A combination therapy according to claim 9, wherein the further anti-cancer therapy is an oncolytic virus.
19. A combination therapy according to claim 18, wherein the oncolytic virus encodes therapeutic gene encoding material selected from the group consisting of an RNAi sequence, an antibody or antigen binding fragment thereof, chemokines, cytokines, immunomodulator and enzymes.