ONCOLYTIC ADENOVIRUSES ARMED WITH HETEROLOGOUS GENES

The present disclosure relates to a modified adenovirus, for example armed with at least one transgene including a therapeutic and/or reporter transgene, in particular a group B virus, such as a serotype 11 adenovirus or a chimeric virus with the fibre, penton and hexon of Ad11, a composition, such as a pharmaceutical formulation comprising the virus, use of the virus and virus formulations, particularly in treatment, especially in the treatment of cancer. The disclosure also extends to processes for preparing the virus.

BACKGROUND

Replication deficient adenoviruses vectors have been investigated for a number of years for the delivery of transgenes. Mostly the genes have been inserted in the E1 region and/or the E3 region because these regions of the viral genome are non-essential for vectors.

Surprisingly relatively little work has been done on alternative locations for inserting transgenes in the adenovirus genome. In addition most of the work has been performed in Ad5.

A new generation of replication competent oncolytic adenoviruses is currently in the clinic. These viruses do not require complementing cell lines to replicate. E1 is an essential region to viral replication and whilst the E3 region in theory can be used as a location to insert a transgene, it would be useful to be able to insert a transgene in more than this location. However, care has to be taken not to disrupt the virus life cycle and/or advantageous viral properties, such as the therapeutic properties of the virus.

Enadenotucirev (EnAd) is a chimeric oncolytic adenovirus, formerly known as EnAd (WO2005/118825), with fibre, penton and hexon from Ad11p, hence it is a subgroup B virus. It has a chimeric E2B region, which comprises DNA from Ad11p and Ad3. Almost all of the E3 region and part of the E4 region is deleted in EnAd. Therefore, it has significant space in the genome to accommodate additional genetic material whilst remaining viable. Furthermore, because EnAd is a subgroup B adenovirus, pre-existing immunity in humans is less common than, for example, Ad5. Other examples of chimeric oncolytic viruses with Ad11 fibre, penton and hexon include OvAd1 and OvAd2 (see WO2006/060314).

EnAd seems to preferentially infect tumour cells, replicates rapidly in these cells and causes cell lysis. This, in turn, can generate inflammatory immune responses thereby stimulating the body to also fight the cancer. Part of the success of EnAd is hypothesised to be related to the fast replication of the virus *in vivo*.

Whilst EnAd selectively lyses tumour cells, it may be possible to introduce further beneficial properties, for example increasing the therapeutic activity of the virus or reducing side-effects of the virus by arming it with transgenes, such as a transgene which encodes a cell signalling protein or an antibody, or a transgene which encodes an entity which stimulates a cell signalling protein(s).

one embodiment SEQ ID NO: 11 is interrupted, for example by a transgene. In embodiment SEQ ID NO: 11 is uninterrupted. In one embodiment B_Y does not comprise a restriction site. In one embodiment B_Y is a bond. In one embodiment B_Y comprises or consists of one or more transgenes.

In one embodiment B_X and B_Y each comprises a restriction site, for example 1, 2, 3 or 4 restriction sites, such as 1 or 2. In one embodiment B_X and B_Y each comprises at least one transgene, for example 1 or 2 transgenes. In one embodiment B_X and B_Y each comprises at least one transgene, for example 1 or 2 transgenes and one or more restriction sites, for example 2 or 3 restriction sites, in particular where the restriction sites sandwich a gene or the DNA sequence comprising the genes to allow it to be specifically excised from the genome and/or replaced. Alternatively the restriction sites may sandwich each gene, for example when there are two transgenes three different restriction sites are required to ensure that the genes can be selectively excised and/or replaced. In one embodiment one or more, for example all the transgenes are in the form a transgene cassette. In one embodiment B_X and B_Y comprises SEQ ID NO: 10 and SEQ ID NO: 11 respectively. In one embodiment B_X and B_Y do not comprise a restriction site. In one embodiment B_X is a bond and B_Y is not a bond. In one embodiment B_Y is a bond and B_X is not a bond.

In one embodiment the transgene is located in B_X. In one embodiment the transgene or transgene cassette is located in B_Y. In one embodiment a transgene or transgene cassette is located in B_X and B_Y, for example the transgenes may be the same or different, in each location.

Advantageously, the transgene in the present virus constructs is/are inserted in a location that is removed from the early genes because this reduces the likelihood of affecting virus gene expression or speed of replication.

In one independent aspect there is provided a replication competent oncolytic adenovirus of serotype 11 or virus-derivative thereof wherein the fibre, hexon and capsid are serotype 11, wherein the virus genome comprises a DNA sequence encoding a therapeutic antibody or antibody-binding fragment, said DNA sequence under the control of a promoter endogenous to the adenovirus selected from consisting of E4 and the major late promoter, such that the transgene does not interfere with virus replication, for example wherein the DNA sequence encoding the therapeutic antibody or antibody-binding fragment is under the control of the E4 promoter or alternatively under the control of the major late promoter, in particular wherein the DNA sequence encoding an antibody or antibody-binding fragment in located after L5 in the virus genome sequence (i.e. towards the 3' end of the virus sequence). Advantageously using an endogenous promoter maximises the amount of space available for inserting transgenes.

Advantageously, when under the control of these promoters the virus remains replication competent and is also able to express the antibody as a full length antibody or a suitable binding fragment or other protein. Thus the antibody or other protein of choice will be expressed by the cancer

ii. an internal ribosome entry (IRES) DNA sequence;

iii. a DNA sequence encoding a high self-cleavage efficiency 2A peptide;

iv. a DNA sequence encoding a polyadenylation sequence, and

v. combinations of the same.

Thus in one embodiment the transgene cassette comprises i) or ii) or iii) or iv).

In one embodiment the transgene cassette comprises i) and ii), or i) and iii), or i) and iv), or ii) and iii), or ii) and iv), or iii) and iv).

In one embodiment the transgene cassette comprises i) and ii) and iii), or i) and ii) and iv), or i) and iii) and iv), or ii) and iii) and iv).

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In one embodiment the transgene or transgene cassette comprises a Kozak squence, which assists in the translation of mRNA, for example at the start of a protein coding sequence.

In one embodiment the virus is replication competent.

In one embodiment the virus is replication deficient, i.e. is a vector.

Also provided is a composition comprising a virus or vector according to the present disclosure, in particular a pharmaceutical composition, for example comprising an adenovirus according to the disclosure and a pharmaceutically acceptable excipient.

The present disclosure further relates to an adenovirus or composition according to the disclosure for use in treatment, for example for use in the treatment of cancer.

The disclosure also relates to a method of treatment comprising administering a therapeutically effective amount of a virus as described herein or a composition comprising the same to a patient in need thereof, in particular a human patient.

DETAILED DESCRIPTION

Transgene as employed herein refers to a gene that has been inserted into the genome sequence, which is a gene that is unnatural to the virus (exogenous) or not normally found in that particular location in the virus. Examples of transgenes are given below. Transgene as employed herein also includes a functional fragment of the gene that is a portion of the gene which when inserted is suitable to perform the function or most of the function of the full-length gene.

Transgene and coding sequence are used interchangeably herein in the context of inserts into the viral genome, unless the context indicates otherwise. Coding sequence as employed herein means, for example a DNA sequence encoding a functional RNA, peptide, polypeptide or protein. Typically the coding sequence is cDNA for the transgene that encodes the functional RNA, peptide, polypeptide or protein of interest. Functional RNA, peptides, polypeptide and proteins of interest are described below.

Clearly the virus genome contains coding sequences of DNA. Endogenous (naturally occurring genes) in the genomic sequence of the virus are not considered a transgene, within the context of the

present specification unless then have been modified by recombinant techniques such as that they are in a non-natural location or in a non-natural environment.

In one embodiment transgene as employed herein refers to a segment of DNA containing a gene or cDNA sequence that has been isolated from one organism and is introduced into a different organism i.e. the virus of the present disclosure. In one embodiment this non-native segment of DNA may retain the ability to produce functional RNA, peptide, polypeptide or protein.

Thus in one embodiment the transgene inserted encodes a human or humanised protein, polypeptide or peptide.

In one embodiment the transgene inserted encodes a non-human protein, polypeptide or peptide (such as a non-human mammalian protein, polypeptide or peptide) or RNA molecule, for example from a mouse, rat, rabbit, camel, llama or similar. Advantageously, the viruses of the present disclosure allow the transgenes to be transported inside the cancerous cell. Thus, responses generated by the human patient to a non-human sequence (such as a protein) can be minimised by this intra-cellular deliver

A DNA sequence may comprise more than one transgene, for example, 1, 2, 3 or 4 transgenes, such as 1 or 2.

A transgene cassette may comprise more than one transgene, for example, 1, 2, 3 or 4 transgenes, such as 1 or 2.

In one or more embodiments the cassette is arranged as shown in the one or more of the Figures or the examples.

Transgene cassette as employed herein refers to a DNA sequence encoding one or more transgenes in the form of one or more coding sequences and one or more regulatory elements.

A transgene cassette may encode one or more monocistronic and/or polycistronic mRNA sequences.

In one embodiment the transgene or transgene cassette encodes a monocistronic or polycistronic mRNA, and for example the cassette is suitable for insertion into the adenovirus genome at a location under the control of an endogenous promoter or exogenous promoter or a combination thereof.

Monocistronic mRNA as employed herein refers to an mRNA molecule encoding a single functional RNA, peptide, polypeptide or protein.

In one embodiment the transgene cassette encodes monocistronic mRNA.

In one embodiment the transgene cassette in the context of a cassette encoding monocistronic mRNA means a segment of DNA optionally containing an exogenous promoter (which is a regulatory sequence that will determine where and when the transgene is active) or a splice site (which is a

immunmodulators, enzymes (for example capable of converting pro-drug in the active agent) and an RNAi molecule.

Peptide as employed herein refers to an amino acid sequence of 2 to 50 residues, for example 5 to 20 residues. Polypeptide as employed herein refers to an amino acid sequence of more than 50 residues without tertiary structure, in particular without secondary and tertiary structure. Protein refers to an amino acid sequence of more than 50 residues, with secondary and/or tertiary structure, in particular with second and tertiary structure.

In one embodiment the coding sequence encodes a therapeutic RNA, therapeutic peptide, therapeutic polypeptide or therapeutic protein (i.e. is a therapeutic gene).

Therapeutic gene as employed herein means a gene that encodes an entity that may be useful in the treatment, amelioration or prevention of disease, for example the gene expresses a therapeutic protein, polypeptide, peptide or RNA, which at least slows down, halts or reverses the progression of a disease, such as cancer.

In one embodiment the entity encoded by the transgene when transcribed or translated in a cell, such as a cancer cell, increases production of danger signals by the cell. “Danger signals” as employed herein refers to a variety of molecules produced by cells undergoing injury, stress or non-apoptotic death that act as alarm signals, for example by stimulating cells of the innate immune system to respond directly as well as serving to enhance activation of cells of the adaptive immune system.

It is known that the microenvironment of tumours often changes such that natural human immune responses are down regulated. Thus the ability to reimm-start the immune responses from within the tumour is potentially very interesting in the treatment of cancer.

In one embodiment the encoded therapeutic peptide or protein is designed to be secreted into the extracellular environment. In one embodiment the functional RNA, peptide, polypeptide or protein, such as the antibody is released into the external microenvironment of the cell, for example into the culture supernatant, or *in vivo*: tissue, stroma, circulation, blood and/or lymphatic system.

In one embodiment the peptide, polypeptide or protein, encoded by the transgene, comprises a signal sequence. Signal peptide as employed herein refers to a short 13-36 residue peptide sequence located at the N-terminal of proteins which assist the entry of the protein into the secretory pathway for secretion or membrane expression. In one embodiment the leader sequence (signal peptide) has the amino acid sequence of SEQ ID NO: 21 or 22.

In another embodiment the encoded therapeutic peptide or protein, such as an antibody is designed to be expressed as a membrane-anchored form in the surface membrane of the cell, for example by including encoding a transmembrane domain in the protein or a site for attachment of a lipid membrane anchor.