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(54) Title: SIMIAN ADENOVIRUS NUCLEIC ACID- AND AMINO ACID-SEQUENCES, VECTORS CONTAINING SAME, AND USES THEREOF

(57) Abstract: The present invention relates to novel adenovirus strains with an improved seroprevalence. In one aspect, the present invention relates to isolated polypeptides of adenoviral capsid proteins such as hexon, penton and fiber protein and fragments thereof and polynucleotides encoding the same. Also provided is a vector comprising the isolated polynucleotide according to the invention and adenoviruses comprising the isolated polynucleotides or polypeptides according to the invention and a pharmaceutical composition comprising said vector, adenovirus, polypeptide and/or polynucleotide. The invention also relates to the use of the isolated polynucleotides, the isolated polypeptides, the vector, the adenoviruses and/or the pharmaceutical composition for the therapy or prophylaxis of a disease.

SIMIAN ADENOVIRUS NUCLEIC ACID- AND AMINO ACID-SEQUENCES, VECTORS CONTAINING SAME, AND USES THEREOF

The present invention relates to novel adenovirus strains with an improved seroprevalence. In one aspect, the present invention relates to isolated polypeptides of adenoviral capsid proteins such as hexon, penton and fiber protein and fragments thereof and polynucleotides encoding the same. Also provided is a vector comprising the isolated polynucleotide according to the invention and adenoviruses comprising the isolated polynucleotides or polypeptides according to the invention and a pharmaceutical composition comprising said vector, adenovirus, polypeptide and/or polynucleotide. The invention also relates to the use of the isolated polynucleotides, the isolated polypeptides, the vector, the adenoviruses and/or the pharmaceutical composition for the therapy or prophylaxis of a disease.

BACKGROUND OF THE INVENTION

The adenoviruses (Ads) comprise a large family of double-stranded DNA viruses found in amphibians, avians, and mammals which have a nonenveloped icosahedral capsid structure (Straus, Adenovirus infections in humans; *The Adenoviruses*, 451-498, 1984; Hierholzer etal.,J. Infect.Dis.,158 : 804-813,1988; Schnurr and Dondero, *Intervirology.*, 36: 79-83,1993 ; Jong et al., *J. Clin. Microbiol.*, 37 : 3940-3945: 1999). In contrast to retroviruses, adenoviruses can transduce numerous cell types of several mammalian species, including both dividing and nondividing cells, without integrating into the genome of the host cell.

Generally speaking, adenoviral DNA is typically very stable and remains episomal (e. g., extrachromosomal), unless transformation or tumorigenesis has occurred. In addition, adenoviral vectors can be propagated to high yields in well-defined production systems which are readily amenable to pharmaceutical scale production of clinical grade compositions. These characteristics and their well-characterized molecular genetics make recombinant adenoviral vectors good candidates for use as vaccine carriers. The production of recombinant adenoviral vectors may rely on the use of a packaging cell line which is capable of complementing the functions of adenoviral gene products that have been either deleted or engineered to be nonfunctional.

Presently, two well-characterized human subgroup C adenovirus serotypes (i. e., hAd2 and hAd5) are widely used as the sources of the viral backbone for most of the adenoviral vectors that are used for gene therapy. Replication-defective human adenoviral vectors have also

been tested as vaccine carriers for the delivery of a variety of immunogens derived from a variety of infectious agents. Studies conducted in experimental animals (e. g. rodents, canines and nonhuman primates) indicate that recombinant replication-defective human adenoviral vectors carrying transgenes encoding immunogens as well as other antigens elicit both humoral and cell-mediated immune responses against the transgene product. Generally speaking, investigators have reported success using human adenoviral vectors as vaccine carriers in nonhuman experimental systems by either using immunization protocols that utilizes high doses of recombinant adenoviral vectors that are predicted to elicit immune responses; or by using immunization protocols which employ the sequential administration of adenoviral vectors that are derived from different serotypes but which carry the same transgene product as boosting immunizations (Mastrangeli, et. al., Human Gene Therapy, 7: 79-87 (1996).

Viral vectors based on human adenovirus type 5 (Ad5) have been developed for different gene therapy and vaccine applications. Although Ad5-based vectors are extremely efficient in animal models, the presence of a pre-existing immunity in humans against Ad5 wild type virus has been demonstrated in clinical trials to reduce the efficiency of gene transduction. In particular, a clear reduction of the immunization efficiency was demonstrated in subjects with titers of neutralizing antibodies over 200 enrolled in vaccine clinical trial based on Ad5 vectors. The most extensive characterization of an Ad5 vectored vaccine was obtained in the HIV vaccine STEP trial conducted by Merck (Moore JP et al. Science. 2008 May 9; 320(5877):753-5). The vaccine study was based on the co-injection of 3 Ad5 vectors expressing different HIV antigens as proof of concept study in subjects with high risk of HIV infection. Surprisingly, the data revealed an increase of HIV infection rate in vaccinated subjects with anti-Ad5 pre-existing immunity rather then a protective effect. Although the mechanism of this paradoxical observation is not clear yet, the results raised additional questions on the safety and efficiency of vectors based on adenovirus of human origin for vaccine application in healthy subjects. Taken together all results obtained so far in different vaccine and gene therapy clinical trials such as the trials with Ad5 vectors increased the need for an adenovirus characterized in a very low or absent pre-existing immunity in humans.

SUMMARY OF THE INVENTION

In a first aspect the present invention provides an isolated polynucleotide that encodes an adenoviral fiber protein or a functional derivative thereof and that is selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide having the amino acid sequence according to any of SEQ ID NOs: 14-19, 50 and 53;

(b) a polynucleotide encoding the functional derivative of a polypeptide according to any of SEQ ID NOs: 14-19, 50 and 53, wherein said functional derivative comprises the deletion, insertion and/or substitution of one or more amino acid residues; and

(c) a polynucleotide encoding a functional derivative having an amino acid sequence which is at least 85% identical over its entire length to the amino acid sequence of any of SEQ ID NOs: 14-19, 50 and 53.

In a further aspect the present invention relates to an isolated polynucleotide that encodes an adenoviral hexon protein or a functional derivative thereof and that is selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide having the amino acid sequence according to any of SEQ ID NOs: 20-25, 51 and 54;

(b) a polynucleotide encoding the functional derivative of a polypeptide according to any of SEQ ID NOs: 20-25, 51 and 54, wherein said functional derivative comprises the deletion, insertion and/or substitution of one or more amino acid residues; and

(c) a polynucleotide encoding a functional derivative having an amino acid sequence which is at least 95% identical over its entire length to the amino acid sequence of any of SEQ ID NOs: 20-25, 51 and 54.

Also provided is an isolated polynucleotide that encodes an adenoviral penton protein or a functional derivative thereof and that is selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide having the amino acid sequence according to any of SEQ ID NOs: 26-31, 52 and 55;

(b) a polynucleotide encoding the functional derivative of a polypeptide according to any of SEQ ID NOs: 26-31, 52 and 55, wherein said functional derivative comprises the deletion, insertion and/or substitution of one or more amino acid residues; and

(c) a polynucleotide encoding a functional derivative having an amino acid sequence which is at least 85% identical over its entire length to the amino acid sequence of any of SEQ ID NOs: 26-31, 52 and 55.

The invention also relates to a polynucleotide comprising at least one of the isolated polynucleotide according to the invention as outlined above. The invention further provides an isolated adenoviral capsid polypeptide encoded by the isolated polynucleotide according to the invention or a functional derivative thereof.

In a further aspect the invention provides a vector comprising the isolated polynucleotide according to the invention.

Also provided is a recombinant adenovirus, preferably a replication-incompetent adenovirus, comprising an isolated polynucleotide according to the invention and/or at least one isolated adenoviral capsid polypeptide according to the invention.

A further aspect of the invention is a composition comprising an adjuvant and at least one of the following (i) through (iv):

(i) one or more isolated adenoviral capsid polypeptides according to the invention;

(ii) an isolated polynucleotide according to the invention;

(iii) a vector according to the invention;

(iv) a recombinant adenovirus according to the invention;

and, optionally, a pharmaceutically acceptable excipient.

The invention further relates to a cell comprising at least one of the following:

(i) one or more isolated adenoviral capsid polypeptides according to the invention;

(ii) an isolated polynucleotide according to the invention;

(iii) a vector according to the invention;

(iv) a recombinant adenovirus according to the invention.

A further aspect of the invention relates to the use of an isolated adenoviral capsid polypeptide according to the invention; an isolated polynucleotide according to the invention; a vector according to the invention; a recombinant adenovirus according to the invention; and/or the composition according to the invention for the therapy or prophylaxis of a disease.

DETAILED DESCRIPTION OF THE INVENTION

Before the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

Preferably, the terms used herein are defined as described in "A multilingual glossary of biotechnological terms: (IUPAC Recommendations)", Leuenberger, H.G.W, Nagel, B. and Klbl, H. eds. (1995), Helvetica Chimica Acta, CH-4010 Basel, Switzerland) and as described in "Pharmaceutical Substances: Syntheses, Patents, Applications" by Axel Kleemann and Jurgen Engel, Thieme Medical Publishing, 1999; the "Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals", edited by Susan Budavari et al., CRC Press, 1996, and the United States

Pharmacopeia-25/National Formulary-20, published by the United States Pharmcopeial Convention, Inc., Rockville Md., 2001.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated feature, integer or step or group of features, integers or steps but not the exclusion of any other feature, integer or step or group of integers or steps. In the following passages different aspects of the invention are defined in more detail. Each aspect so defined may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous.

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, etc.), whether *supra* or *infra*, are hereby incorporated by reference in their entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

In the following, some definitions of terms frequently used in this specification are provided. These terms will, in each instance of its use, in the remainder of the specification have the respectively defined meaning and preferred meanings.

Generally speaking, the adenoviral genome is well characterized. There is general conservation in the overall organization of the adenoviral genome with respect to specific open reading frames being similarly positioned, e.g. the location of the E1A, E1B, E2A, E2B, E3, E4, LI, L2, L3, L4 and L5 genes of each virus. Each extremity of the adenoviral genome comprises a sequence known as an inverted terminal repeat (ITRs), which is necessary for viral replication. The virus also comprises a virus-encoded protease, which is necessary for processing some of the structural proteins required to produce infectious virions. The structure of the adenoviral genome is described on the basis of the order in which the viral genes are expressed following host cell transduction. More specifically, the viral genes are referred to as early (E) or late (L) genes according to whether transcription occurs prior to or after onset of DNA replication. In the early phase of transduction, the E1A, E1B, E2A, E2B, E3 and E4 genes of adenovirus are expressed to prepare the host cell for viral replication. During the late phase of infection, expression of the late genes L1-L5, which encode the structural components of the virus particles are activated.

The following Table 1 provides an overview over the sequences referred to herein:

Table 1

Designation/Strain	SEQ ID NO:	Protein	Polynucleotide
HIV gag	1		HIV gag
TLR9 agonist	2		TLR9 agonist
HVR7 primer1	3		HVR7 primer1
HVR7 primer2	4		HVR7 primer2
HVR1-6fd	5		HVR1-6fd
HVR1-6rev	6		HVR1-6rev
PanAd1 left end P1	7		PanAd1 left end P1
PanAd1 left end P2	8		PanAd1 left end P2
PanAd1 right end P1	9		PanAd1 right end P1
PanAd1 right end P2	10		PanAd1 right end P2
pIX P1	11		pIX P1
pIX P2	12		pIX P2
Bonobo Adenovirus type 1 (PanAd1). Complete genome	13		Bonobo Adenovirus type 1 (PanAd1). Complete genome
ChAd55	14	Fiber	
ChAd73	15	Fiber	
ChAd83	16	Fiber	
ChAd146	17	Fiber	
ChAd147	18	Fiber	
PanAd1	19	Fiber	
ChAd55	20	Hexon	
ChAd73	21	Hexon	
ChAd83	22	Hexon	
ChAd146	23	Hexon	
ChAd147	24	Hexon	
PanAd1	25	Hexon	
ChAd55	26	Penton	
ChAd73	27	Penton	
ChAd83	28	Penton	
ChAd146	29	Penton	
ChAd147	30	Penton	
PanAd1	31	Penton	
ChAd55	32		Fiber
ChAd73	33		Fiber
ChAd83	34		Fiber
ChAd146	35		Fiber
ChAd147	36		Fiber
PanAd1	37		Fiber
ChAd55	38		Hexon
ChAd73	39		Hexon
ChAd83	40		Hexon
ChAd146	41		Hexon
ChAd147	42		Hexon
PanAd1	43		Hexon
ChAd55	44		Penton
ChAd73	45		Penton
ChAd83	46		Penton
ChAd146	47		Penton
ChAd147	48		Penton
PanAd1	49		Penton

PanAd2	50	Fiber	
PanAd2	51	Hexon	
PanAd2	52	Penton	
PanAd3	53	Fiber	
PanAd3	54	Hexon	
PanAd3	55	Penton	
PanAd2	56		Fiber
PanAd2	57		Hexon
PanAd2	58		Penton
PanAd3	59		Fiber
PanAd3	60		Hexon
PanAd3	61		Penton
Bonobo Adenovirus type 2 (PanAd2). Complete genome	62		Bonobo Adenovirus type 2 (PanAd2). Complete genome
Bonobo Adenovirus type 3 (PanAd3). Complete genome	63		Bonobo Adenovirus type 3 (PanAd3). Complete genome
Ad5 E4 ORF6 coding sequence	64		Ad5 E4 ORF6 coding sequence
ChAd83 Complete genome	65		ChAd83 Complete genome

As used herein, the term "isolated" refers to a molecule which is substantially free of other molecules with which it is naturally associated with. An isolated molecule is thus free of other molecules that it would encounter or contact in a living animal in nature, i.e. outside an experimental setting.

As used herein, the term "protein", "peptide", "polypeptide", "peptides" and "polypeptides" are used interchangeably throughout. These terms refers to both naturally occurring peptides, e.g. naturally occurring proteins and synthesized peptides that may include naturally or non-naturally occurring amino acids. Peptides can be also chemically modified by modifying a side chain or a free amino or carboxy-terminus of a natural or non-naturally occurring amino acid. This chemical modification includes the addition of further chemical moieties as well as the modification of functional groups in side chains of the amino acids, such as a glycosylation. A peptide is a polymer preferably having at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or at least 100 amino acids, most preferably at least 8 or at least 30 amino acids. As the polypeptides and proteins disclosed herein are derived from adenovirus, it is preferred that the molecular mass of an isolated polypeptide or protein as used herein does not exceed 200 kDa.

The term "vector" as used herein includes any vectors known to the skilled person including plasmid vectors, cosmid vectors, phage vectors such as lambda phage, viral vectors such as adenovirus (Ad) vectors (e.g., non-replicating Ad5, Ad11, Ad26, Ad35, Ad49, ChAd3, ChAd4, ChAd5, ChAd7, ChAd8, ChAd9, ChAd10, ChAd11, ChAd16, ChAd17, ChAd19,

ChAd20 , ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd63 and ChAd82 vectors or replication-competent Ad4 and Ad7 vectors known from the prior art, e.g. WO 2005/071093 A2), adeno-associated virus (AAV) vectors (e.g., AAV type 5), alphavirus vectors (e.g., Venezuelan equine encephalitis virus (VEE), sindbis virus (SIN), semliki forest virus (SFV), and VEE-SIN chimeras), herpes virus vectors, measles virus vectors, pox virus vectors (e.g., vaccinia virus, modified vaccinia virus Ankara (MVA), NYVAC (derived from the Copenhagen strain of vaccinia), and avipox vectors: canarypox (ALVAC) and fowlpox (FPV) vectors), and vesicular stomatitis virus vectors, viral like particles, or bacterial spores. A vector also includes expression vectors, cloning vectors and vectors that are useful to generate recombinant adenoviruses in host cells.

The term "expression cassette" refers to a nucleic acid molecule which comprises at least one nucleic acid sequence that is to be expressed, along with its transcription and translation control sequences. Changing the expression cassette will cause the vector in which it is incorporated to direct the expression of a different sequence or combination of sequences. Because of the restriction sites being preferably engineered to be present at the 5' and 3' ends, the cassette can be easily inserted, removed, or replaced with another cassette. Preferably, an expression cassette includes cis-regulating elements for efficient expression of a given gene, such as promoter, initiation-site and/or polyadenylation-site, as further described below.

The term "antibody" refers to both monoclonal and polyclonal antibodies, i.e., any immunoglobulin protein or portion thereof which is capable of binding an antigen or hapten. Antigen-binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. In some embodiments, antigen-binding portions include Fab, Fab', $F(ab')_2$, Fd, Fv, dAb, and complementarity determining region (CDR) variants, single-chain antibodies (scFv), chimeric antibodies, humanized antibodies, diabodies, and polypeptides that contain at least a portion of an antibody that is sufficient to confer specific antigen binding to the polypeptide.

The administration of an immunogen / antigen for inducing/generating an immune response in a mammal in the context of the present invention is termed "priming", and the administration of an immunogen/ antigen for enhancing an immune response against said immunogen/ antigen, e.g. a particular pathogen (such as a virion or a virus pathogen, an antigen of a pathogenic bacterium or a tumorantigen) in a mammal is termed "boosting". The phrase "heterologous prime-boost" means that the vector for inducing/generating an immune response (priming) in a mammal and the vector for enhancing the immune response (boosting) in a mammal are different. "Heterologous prime-boost" is useful if a subject, e.g. patient has developed antibodies against a first vector and a boosting is required. Thus, in a preferred

embodiment of heterologous prime-boost two different adenoviruses may be used, e.g. for vaccination and/or gene therapy. In this context, a first and a second adenovirus are sufficiently different, if the antibody response induced during priming by the first adenovirus does not prevent more than 70% or preferably more than 80% of the second adenovirus particles administered for boosting from entering the nucleus of cells of the animal that has been subjected to priming and boosting.

The term "replication-competent" recombinant adenovirus (AdV) refers to an adenovirus which can replicate in a host cell in the absence of any recombinant helper proteins comprised in the cell. Preferably, a "replication-competent" adenovirus comprises the following intact or functional essential early genes: E1A, E1B, E2A, E2B, E3 and E4. Wild type adenoviruses isolated from a particular animal will be replication competent in that animal.

The term "replication-defective" recombinant AdV refers to an adenovirus that has been rendered to be incapable of replication because it has been engineered to comprise at least a functional deletion, i.e. a deletion which impairs the function of a gene without removing it entirely, e.g. introduction of artificial stop codons, deletion or mutation of active sites or interaction domains, mutation or deletion of a regulatory sequence of a gene etc, or a complete removal of a gene encoding a gene product that is essential for viral replication, such as one or more of the adenoviral genes selected from E1, E2, E3 and E4. The recombinant chimpanzee adenoviral vectors of the invention are preferably replication-defective.

The term "identity" or "identical" in the context of polynucleotide, polypeptide or protein sequences refers to the number of residues in the two sequences that are identical when aligned for maximum correspondence. Specifically, the percent sequence identity of two sequences, whether nucleic acid or amino acid sequences, is the number of exact matches between two aligned sequences divided by the length of the shorter sequence and multiplied by 100. Alignment tools that can be used to align two sequences are well known to the person skilled in the art and can, for example, be obtained on the World Wide Web, e.g., ClustalW (www.ebi.ac.uk/clustalw) or Align (http://www.ebi.ac.uk/emboss/align/index.html). The alignments between two sequences may be carried out using standard settings, for Align EMBOSS::needle preferably: Matrix: Blosum62, Gap Open 10.0, Gap Extend 0.5. Those skilled in the art understand that it may be necessary to introduce gaps in either sequence to produce a satisfactory alignment. The "best sequence alignment" between two polypeptides is defined as the alignment that produces the largest number of aligned identical residues.

Adenoviruses

An adenovirus (Ad) is a non-enveloped, icosahedral virus that has been identified in several avian and mammalian hosts. Human adenoviruses (hAds) belong to the Mastadenovirus genus which includes all known human and many Ads of animal (e. g., bovine, porcine, canine, murine, equine, simian and ovine) origin. Human adenoviruses are generally divided into six subgroups (A-F) based on a number of biological, chemical, immunological and structural criteria which include hemagglutination properties of rat and rhesus monkey erythrocytes, DNA homology, restriction enzyme cleavage patterns, percentage G+C content and oncogenicity (Straus, 1984, in *The Adenoviruses*, ed. H. Ginsberg, pps.451-498, New York : Plenus Press, and Horwitz, 1990; in *Virology*, eds. B. N. Fields and D. M. Knipe, pps. 1679-1721).

The adenoviral virion has an icosahedral symmetry and, depending on the serotype, a diameter of 60-90 nm. The icosahedral capsid comprises three major proteins, hexon (II), penton base (III) and a knobbed fiber (IV) protein (W. C. Russel, J. Gen.Virol., 81: 2573-2604 (2000)). One aspect of the preexisting immunity that is observed in humans is humoral immunity, which can result in the production and persistence of antibodies that are specific for adenoviral proteins. The humoral response elicited by adenovirus is mainly directed against the three major structural proteins: hexon, penton and fiber.

To date, 51 distinct human adenovirus serotypes have been recognized and grouped into subgroups on the basis of their hemagglutination properties and biophysical and biochemical criteria. Published reports have established that titers comprising antibodies against multiple serotypes are common (Dambrosio, E. (1982) J. Hyg. (London) 89: 209-219) and that a substantial portion of the titers have neutralizing activity.

As mentioned, recombinant adenoviruses are useful in gene-therapy and as vaccines. Viral vectors based on chimpanzee adenovirus represent an alternative to the use of human derived Ad vectors for the development of genetic vaccines (Farina SF, J Virol. 2001 Dec;75(23):11603-13.; Fattori E, Gene Ther. 2006 Jul;13(14):1088-96). Adenoviruses isolated from chimpanzees are closely related to adenoviruses isolated from humans as demonstrated by their efficient propagation in cells of human origin. However, since human and chimp adenoviruses are close relatives, a serologic cross reactivity between the two virus species can be expected.

This presumption has been confirmed when chimpanzee adenoviruses were isolated and characterized. Nevertheless, adenovirus isolates from chimpanzees showed a reduced cross reactivity with the common serotypes of human adenovirus epitopes. Thus, a chimpanzee adenovirus (also abbreviated herein as "ChAd" for common chimpanzee adenovirus and "PanAd" for bonobo chimpanzee adenovirus) provides a basis for reducing the adverse effects

associated with the preexisting immunity in humans to common serotypes of human adenoviruses. However, a low to intermediate neutralizing titer against chimp adenoviruses isolated so far is detected in subsets of human sera and, thus, all known serotypes of chimpanzee adenoviruses are still neutralized by human blood sera to some degree.

The present invention comprises the unexpected finding that novel chimpanzee adenovirus strains could be isolated, namely ChAd55, ChAd73, ChAd83, ChAd146, ChAd147 isolated from the Common Chimpanzee (*Pan troglodytes*) and PanAd1, PanAd2 and PanAd3 isolated from bonobos (*Pan paniscus*). All these novel strains show *no* measurable seroprevalence in humans, i.e. these adenovirus strains represent an exception among chimpanzee adenoviruses described so far in that all human sera tested completely negative for the presence of neutralizing antibodies. In this context, a neutralizing antibody refers to an antibody that binds to an epitope of the adenovirus and prevents it from producing a productive infection in a host cell or prevents the transduction of a target cell with a replication incompentent vector expressing a transgene, e.g. the adenovirus DNA is capable of entering a host cell. While neutralizing antibodies were observed for all prior-art chimpanzee-derived adenoviruses, the novel adenovirus types ChAd55, ChAd73, ChAd83, ChAd146, ChAd147 PanAd1, PanAd2 and PanAd3 are characterized by a complete absence of preexisting neutralizing antibody in humans directed against these adenovirus types. Thus, these adenoviruses provide a valuable medical tool that can e.g. be used for immunization and/or gene therapy.

As detailed further below, the invention provides, in one aspect, novel sequences of adenovirus capsid proteins that represent the most surface exposed adenovirus epitopes, namely hexon, penton and fiber protein. As already mentioned, no neutralizing antibodies specific for the viruses according to the invention are comprised in human blood sera. Thus, one advantage of the aforementioned novel chimpanzee hexon, penton and fiber protein sequences is that the sequences of these proteins can be used to enhance prior art adenoviruses, which have been engineered for e.g. medical purposes. For example, the capsid proteins or functional fragments thereof of the present invention can be used to e.g. replace/substitute one or more of the major structural capsid proteins or functional fragments thereof, respectively, of a different adenovirus, e.g. a prior art adenovirus, to obtain improved recombinant adenoviruses with a reduced seroprevalence in humans. As the novel adenoviruses of the invention but also adenoviruses which have been re-engineered as described will not encounter any significant inhibitory immune response in humans when administered, their overall transduction efficiency and infectivity will be enhanced. Thus, such improved adenoviruses are expected to be, e.g., more effective vaccines as the entry into host cells and the expression of the antigen cassette will not

be hampered by any significant titer of neutralizing antibodies. In addition, as shown in the examples, a potent immune response against HIV gag was elicited even in naïve mice vaccinated with a recombinant HIV-gag encoding adenovirus that comprises hexon, penton and fiber proteins of the ChAd55, ChAd73, ChAd83, ChAd146, ChAd147, PanAd1, PanAd2 or PanAd3 isolate. The immune response elicited by ChAd55-gag, ChAd73-gag, ChAd83-gag, ChAd146-gag, ChAd147-gag, PanAd1-gag, PanAd2-gag and PanAd3-gag adenoviruses is comparable with the response observed with the most potent vectors developed so far based on recombinant human Ad5 vector of the prior art expressing HIV gag protein (see data of an ELIspot assay in figure 5A, 5B, 5C).

As mentioned before, the humoral response elicited by an adenovirus is mainly directed against the three major adenoviral structural proteins: hexon, penton and fiber, all of which comprise polypeptide sequences that are part of the adenoviral capsid and that are exposed to the outside of the virus particle (see also: Madisch I, et al., J. Virol. 2005 Dec;79(24):15265-76; and also: Madisch I, et al., J Virol. 2007 Aug;81(15):8270-81; and Pichla-Gollon SL, et al., J. Virol. 2007 Feb;81(4):1680-9).

As depicted in the multiple sequence alignment shown in figure 1, the novel adenovirus isolates of the group of PanAd1, PanAd2, PanAd3, ChAd55, ChAd73, ChAd83, ChAd146 and ChAd147 of the present invention share a very similar hexon protein sequence. In the alignment also the hypervariable regions (HVRs) are labeled which occur in loops at the top of the hexon molecule that lie on the exterior of the virion and cover a large amount of its surface (see Jophn J. Rux et. Al, J. of Virology, Sept 2003, vol. 77, no.17). The sequence relatedness of the further capsid proteins fiber and penton of the novel chimpanzee adenoviruses is provided in figures 2 and 3, respectively. All three structural capsid proteins are expected to contribute to the low seroprevalence and can, thus, be used independently from each other or in combination to suppress the affinity of an adenovirus to preexisting neutralizing antibodies, e.g. to manufacture a recombinant chimeric adenovirus with a reduced seroprevalence.

Thus, in a first aspect the invention provides an isolated polynucleotide that encodes an adenoviral fiber protein or a functional derivative thereof and that is selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide having the amino acid sequence according to any of SEQ ID NOs: 14-19, 50 and 53; i.e. SEQ ID NO: 14, 15, 16, 17, 18 , 19, 50 or 53;

(b) a polynucleotide encoding the functional derivative of a polypeptide according to any of SEQ ID NOs: 14-19, 50 and 53, i.e. SEQ ID NO: 14, 15, 16, 17, 18, 19, 50 o 53; wherein said functional derivative comprises the deletion, insertion and/or substitution of one or more amino acid residues; and

(c) a polynucleotide encoding a functional derivative having an amino acid sequence which is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or at least 99%, more preferably at least 85% and most preferable at least 99% identical over its entire length to the amino acid sequence of any of SEQ ID NOs: 14-19, 50 and 53, i.e. SEQ ID NO: 14, 15, 16, 17, 18, 19, 50 or 53.

By "adenoviral fiber protein" is meant the knobbed fiber (IV) protein comprised in an adenovirus. In a preferred embodiment, the isolated polynucleotide comprised in the first aspect of the invention and preferred embodiments thereof described below encodes a fiber protein or a functional derivative thereof that has the same function as a fiber protein or a fragment thereof in an *infectious* adenovirus virion. Thus, a recombinant adenovirus comprising said fiber or functional fiber derivative preferably as a capsid protein is capable of entering a host cell. It can be easily determined if a recombinant adenovirus can enter a host cell. For example, after contacting a host cell with the adenovirus, the recombinant host cell can be washed and lysed and it can be determined whether adenoviral RNA and/or DNA is found in the host cell using, e.g. an appropriate hybridization probe specific for adenoviral RNA and/or DNA. Alternatively or additionally, the host cell after having been brought into contact with the recombinant adenovirus may be washed, lysed and probed with adenovirus specific antibodies, e.g. using a Western blot. In yet another alternative, it is observed, e.g. *in vivo*, whether the host cell expresses a gene product, for example a fluorescent protein upon infection with a recombinant adenovirus that comprises a suitable expression cassette to express the gene product in the host cell.

It is further preferred that the fiber protein and functional derivative thereof has an affinity to an adenoviral penton protein, such as to SEQ ID NOs: 26-31, 52 and/or 55. The average skilled person is well aware of how to test protein-protein affinities. To determine if a first protein is capable of binding a second protein, such as a penton protein of a chimpanzee derived adenovirus, he may use, for example, a genetic yeast two-hybrid assay or a biochemical assay such as a pull-down, an enzyme-linked immunosorbent assay (ELISA), a fluorescence-activated cell sorting (FACS)-based assay or a Plasmon resonance assay. When using pull-down or Plasmon resonance assays, it is useful to fuse at least one of the proteins to an affinity tag such as HIS-tag, GST-tag or other, as is well known in the art of biochemistry. An adenoviral fiber protein in its glycosylated form is further capable of trimerizing. Thus, it is also preferred that the fiber protein or a fragment thereof encoded by the polynucleotide according to the first aspect of the invention is capable of being glycosylated and/or of forming a trimer.

As used throughout this application, the phrase "functional derivative" of a protein or polypeptide generally refers to a modified version of the protein or polypeptide, e.g. one or more

amino acids of the protein or polypeptide may be deleted, inserted, modified and/or substituted. The derivative is functional, if, as mentioned also above, a chimeric adenovirus comprising the functional derivative in its capsid is capable of infecting a host cell. Furthermore, in the context of a "functional derivative", an insertion refers to the insertion of one or more amino acids into the original polypeptide or protein. It is preferred that a functional derivative does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100 amino acid changes (i.e. deleted, inserted, modified and/or substituted amino acids). In another embodiment, it is preferred that not more than 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, or more than 20% (most preferably not more than 5%) of all amino acids of the protein or polypeptide are changed (i.e. are deleted, inserted, modified and/or substituted amino acids). Amino acids of the protein or polypeptide may also be modified, e.g. chemically modified. For example, the side chain or a free amino or carboxy-terminus of an amino acid of the protein or polypeptide may be modified by e.g. glycosylation, amidation, phosphorylation, ubiquitination, e.t.c. The chemical modification can also take place *in vivo*, e.g. in a host-cell, as is well known in the art. For examples, a suitable chemical modification motif, e.g. glycosylation sequence motif present in the amino acid sequence of the protein will cause the protein to be glycosylated. A substitution in a derivative may be a conservative or a non-conservative substitution, preferably a conservative substitution. In some embodiments, a substitution also includes the exchange of a naturally occurring amino acid with a not naturally occurring amino acid. A conservative substitution comprises the substitution of an amino acid with another amino acid having a chemical property similar to the amino acid that is substituted. Preferably, the conservative substitution is a substitution selected from the group consisting of:

(i) a substitution of a basic amino acid with another, different basic amino acid;

(ii) a substitution of an acidic amino acid with another, different acidic amino acid;

(iii) a substitution of an aromatic amino acid with another, different aromatic amino acid;

(iv) a substitution of a non-polar, aliphatic amino acid with another, different non-polar, aliphatic amino acid; and

(v) a substitution of a polar, uncharged amino acid with another, different polar, uncharged amino acid.

A basic amino acid is preferably selected from the group consisting of arginine, histidine, and lysine. An acidic amino acid is preferably aspartate or glutamate. An aromatic amino acid is preferably selected from the group consisting of phenylalanine, tyrosine and tryptophane. A non-polar, aliphatic amino acid is preferably selected from the group consisting of glycine, alanine, valine, leucine, methionine and isoleucine. A polar, uncharged amino acid is preferably selected from the group consisting of serine, threonine, cysteine, proline, asparagine and glutamine. In

contrast to a conservative amino acid substitution, a non-conservative amino acid substitution is the exchange of one amino acid with any amino acid that does not fall under the above-outlined conservative substitutions (i) through (v).

If a functional derivative comprises a deletion, then in the derivative one or several amino acids that are present in the reference polypeptide or protein sequence have been removed. The deletion may, however, not be so extensive that the derivative comprises less than 200 amino acids in total.

Means for determining sequence identity have been described already above. In addition, the determination of percent identity between two sequences can also be determined using the mathematical algorithm of Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90: 5873-5877. Such an algorithm is also incorporated into the BLASTN and BLASTP programs of Altschul et al. (1990) J. Mol. Biol. 215: 403-410. When utilizing BLASTN and BLASTP it is preferred that the default parameters of these programs are used.

As mentioned before, the hyper variable domains of an adenoviral hexon protein are exposed to the outside of the adenovirus. Thus, these regions of the adenoviral capsid can be recognized and bound by neutralizing antibodies. Thus, an adenovirus with a capsid comprising a hexon protein derived from one of the novel adenovirus isolates of the present invention will exhibit an improved, i.e. smaller seroprevalence in humans. Thus, in a second aspect the invention provides an isolated polynucleotide that encodes an adenoviral hexon protein or a functional derivative thereof and that is selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide having the amino acid sequence according to any of SEQ ID NOs: 20-25, 51 and 54, i.e. SEQ ID NO: 20, 21, 22, 23, 24, 25, 51 or 54;

(b) a polynucleotide encoding the functional derivative of a polypeptide according to any of SEQ ID NOs: 20-25, 51 and 54, i.e. SEQ ID NO: 20, 21, 22, 23, 24, 25, 51 or 54 wherein said functional derivative comprises the deletion, insertion and/or substitution of one or more amino acid residues; and

(c) a polynucleotide encoding a functional derivative having an amino acid sequence which is at least 95%, 98%, 99%, 99.5%, 99.9% or at least 99.95%, more preferably at least 98% and most preferable at least 99.95% identical over its entire length to the amino acid sequence of any of SEQ ID NOs: 20-25, 51 and 54, i.e. SEQ ID NO: 20, 21, 22, 23, 24, 25, 51 or 54.

In a preferred embodiment, the isolated polynucleotide comprised in the second aspect of the invention and preferred embodiments thereof described below encodes a hexon protein or a functional derivative thereof that has the same function as a hexon protein or a functional fragment thereof in an *infectious* adenovirus virion. Thus, a recombinant adenovirus comprising

said hexon or functional derivative thereof preferably as a capsid protein is capable of entering a host cell. One suitable method for generating functional derivatives of a hexon protein is described in US Patent 5,922,315, which is incorporated by reference. In this method, at least one loop region of the adenovirus hexon is changed with at least one loop region of another adenovirus serotype. For example, a loop region of a hexon protein of the invention can be used to substitute the corresponding hexon loop of an adenovirus of the prior art to generate an improved hybrid adenovirus. Analogously also derivatives of penton and fiber proteins of the invention can be generated.

In a third aspect, the invention provides an isolated polynucleotide that encodes an adenoviral penton protein or a functional derivative thereof and that is selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide having the amino acid sequence according to any of SEQ ID NOs: 26-31, 52 and 55, i.e. SEQ ID NO: 26, 27, 28, 29, 30, 31, 52 or 55;

(b) a polynucleotide encoding the functional derivative of a polypeptide according to any of SEQ ID NOs: 26-31, 52 and 55, i.e. SEQ ID NO: 26, 27, 28, 29, 30, 31, 52 or 55; wherein said functional derivative comprises the deletion, insertion and/or substitution of one or more amino acid residues; and

(c) a polynucleotide encoding a functional derivative having an amino acid sequence which is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or at least 99%, more preferably at least 85% and most preferable at least 99% identical over its entire length to the amino acid sequence of any of SEQ ID NOs: 26-31, 52 and 55, i.e. SEQ ID NO: 26, 27, 28, 29, 30, 31, 52 or 55.

It is preferred that the penton protein and functional derivative thereof has an affinity to an adenoviral fiber protein, such as to SEQ ID NOs: 14-19, 50 and/or 53. The average skilled person is well aware of how to test protein-protein affinities as described above. By "adenoviral penton protein" is meant the penton base (III) protein comprised in an adenovirus. An adenoviral penton protein is characterized in that it localizes to the corners of the icosahedral symmetry of the capsid. As mentioned, in a preferred embodiment of the polynucleotide of the first, second and/or third aspect of the invention and preferred embodiments thereof described herein below, the polynucleotide encodes one or more polypeptides, wherein a recombinant adenovirus comprising said one or more polypeptides preferably as a capsid protein(s) is capable to infect, i.e. enter a host cell.

In the following, preferred embodiments of the first, second and third aspect of the invention will be specified for each of the novel chimpanzee adenovirus isolates disclosed herein.

Adenovirus ChAd55

In a preferred embodiment of the first aspect of the invention, the isolated polynucleotide encodes an adenoviral fiber protein with an amino acid sequence according to SEQ ID NO: 14 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has an amino acid sequence which is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or at least 99%, more preferably at least 85% and most preferable at least 99% identical over its entire length to the amino acid sequence of SEQ ID NO: 14.

In a preferred embodiment of the second aspect of the invention, the isolated polynucleotide encodes an adenoviral hexon protein with an amino acid sequence according to SEQ ID NO: 20 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has an amino acid sequence which is at least 95%, 98%, 99%, 99.5%, 99.9% or at least 99.95%, more preferably at least 98% identical over its entire length to the amino acid sequence of SEQ ID NO: 20.

In a preferred embodiment of the third aspect of the invention, the isolated polynucleotide encodes an adenoviral penton protein with an amino acid sequence according to SEQ ID NO: 26 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has an amino acid sequence which is at least 98%, 99%, 99.5%, 99.9% or at least 99.95%, more preferably at least 98% and most preferable at least 99.9% identical over its entire length to the amino acid sequence of SEQ ID NO: 26.

In a further aspect the invention relates to a polynucleotide comprising the first, the second, the third, the first and second, the first and third, the second and third or the first, second and third aspect. It is preferred that the polynucleotide comprising this or these polynucleotide(s) comprises other adenoviral genes and nucleotide segments, which are adjacent to the hexon, penton and/or fiber gene in the adenovirus genome, e.g. using the Ad5 genome as a reference. It is preferred that the polynucleotide also comprises sequences required for packaging of the polynucleotide into an adenoviral particle.

Adenovirus ChAd73

In a preferred embodiment of the first aspect of the invention, the isolated polynucleotide encodes an adenoviral fiber protein with an amino acid sequence according to SEQ ID NO: 15 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has an amino acid sequence which is at least 98%, 99% or at least 99.9% more preferably at least 99% and most preferable at least 99.9% identical over its entire length to the amino acid sequence of SEQ ID NO: 15.

In a preferred embodiment of the second aspect of the invention, the isolated polynucleotide encodes an adenoviral hexon protein with an amino acid sequence according to SEQ ID NO: 21 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has an amino acid sequence which is at least 95%, 98%, 99%, 99.5%, 99.9% or at least 99.95%, more preferably at least 98% identical over its entire length to the amino acid sequence of SEQ ID NO: 21.

In a preferred embodiment of the third aspect of the invention, the isolated polynucleotide encodes an adenoviral penton protein with an amino acid sequence according to SEQ ID NO: 27 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has an amino acid sequence which is at least 98%, 99%, 99.5%, 99.9% or at least 99.95%, more preferably at least 98% and most preferable at least 99% identical over its entire length to the amino acid sequence of SEQ ID NO: 27.

In a further aspect the invention relates to a polynucleotide comprising the first, the second, the third, the first and second, the first and third, the second and third or the first, second and third aspect. It is preferred that the polynucleotide comprising this or these polynucleotide(s) comprises other adenoviral genes and nucleotide segments, which are adjacent to the hexon, penton and/or fiber gene in the adenovirus genome, e.g. using the Ad5 genome as a reference. It is preferred that the polynucleotide also comprises sequences required for packaging of the polynucleotide into an adenoviral particle.

Adenovirus ChAd83

In a preferred embodiment of the first aspect of the invention, the isolated polynucleotide encodes an adenoviral fiber protein with an amino acid sequence according to SEQ ID NO: 16 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has the amino acid sequence of SEQ ID NO: 16.

In a preferred embodiment of the second aspect of the invention, the isolated polynucleotide encodes an adenoviral hexon protein with an amino acid sequence according to SEQ ID NO: 22 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has an amino acid sequence which is at least 95%, 98%, 99%, 99.5%, 99.9% or at least 99.95%, more preferably at least 98% identical over its entire length to the amino acid sequence of SEQ ID NO: 22.

In a preferred embodiment of the third aspect of the invention, the isolated polynucleotide encodes an adenoviral penton protein with an amino acid sequence according to SEQ ID NO: 28 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has an amino acid sequence which is at least 98%, 99%, 99.5%, 99.9% or at least 99.95%, more preferably at least 98% and most preferable at least 99% identical over its entire length to the amino acid sequence of SEQ ID NO: 28.

In a most preferred embodiment, the polynucleotide of the invention consists of or comprises a polynucleotide which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% identical and most preferably at least 99% or 100% identical over its entire length to a sequence that consists of SEQ ID NO: 65 or to a sequence that consists of SEQ ID NO: 65 but lacks any of the genomic regions E1A, E1B, E2A, E2B, E3 and/or E4 of SEQ ID NO: 65, most preferably that lacks the genomic regions E1, E3 and E4 of SEQ ID NO: 65.

In a further aspect the invention relates to a polynucleotide comprising the first, the second, the third, the first and second, the first and third, the second and third or the first, second and third aspect. It is preferred that the polynucleotide comprising this or these polynucleotide(s) comprises other adenoviral genes and nucleotide segments, which are adjacent to the hexon, penton and/or fiber gene in the adenovirus genome, e.g. using the ChAd83 genome as set out in

SEQ ID NO: 65. It is preferred that the polynucleotide also comprises sequences required for packaging of the polynucleotide into an adenoviral particle.

Adenovirus ChAd146

In a preferred embodiment of the first aspect of the invention, the isolated polynucleotide encodes an adenoviral fiber protein with an amino acid sequence according to SEQ ID NO: 17 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has the amino acid sequence of SEQ ID NO: 17.

In a preferred embodiment of the second aspect of the invention, the isolated polynucleotide encodes an adenoviral hexon protein with an amino acid sequence according to SEQ ID NO: 23 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has an amino acid sequence which is at least 95%, 98%, 99%, 99.5%, 99.9% or at least 99.95%, more preferably at least 98% identical over its entire length to the amino acid sequence of SEQ ID NO: 23.

In a preferred embodiment of the third aspect of the invention, the isolated polynucleotide encodes an adenoviral penton protein with an amino acid sequence according to SEQ ID NO: 29 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has an amino acid sequence which is at least 98%, 99%, 99.5%, 99.9% or at least 99.95%, more preferably at least 98% and most preferable at least 99% identical over its entire length to the amino acid sequence of SEQ ID NO: 29.

In a further aspect the invention relates to a polynucleotide comprising the first, the second, the third, the first and second, the first and third, the second and third or the first, second and third aspect. It is preferred that the polynucleotide comprising this or these polynucleotide(s) comprises other adenoviral genes and nucleotide segments, which are adjacent to the hexon, penton and/or fiber gene in the adenovirus genome, e.g. using the Ad5 genome as a reference. It is preferred that the polynucleotide also comprises sequences required for packaging of the polynucleotide into an adenoviral particle.

Adenovirus ChAd147

In a preferred embodiment of the first aspect of the invention, the isolated polynucleotide encodes an adenoviral fiber protein with an amino acid sequence according to SEQ ID NO: 18 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has an amino acid sequence which is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or at least 99%, more preferably at least 85% and most preferable at least 90% identical over its entire length to the amino acid sequence of SEQ ID NO: 18.

In a preferred embodiment of the second aspect of the invention, the isolated polynucleotide encodes an adenoviral hexon protein with an amino acid sequence according to SEQ ID NO: 24 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has an amino acid sequence which is at least 95%, 98%, 99%, 99.5%, 99.9% or at least 99.95%, more preferably at least 98% identical over its entire length to the amino acid sequence of SEQ ID NO: 24.

In a preferred embodiment of the third aspect of the invention, the isolated polynucleotide encodes an adenoviral penton protein with an amino acid sequence according to SEQ ID NO: 30 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has an amino acid sequence which is at least 98%, 99%, 99.5%, 99.9% or at least 99.95%, more preferably at least 98% and most preferable at least 99% identical over its entire length to the amino acid sequence of SEQ ID NO: 30.

In a further aspect the invention relates to a polynucleotide comprising the first, the second, the third, the first and second, the first and third, the second and third or the first, second and third aspect. It is preferred that the polynucleotide comprising this or these polynucleotide(s) comprises other adenoviral genes and nucleotide segments, which are adjacent to the hexon, penton and/or fiber gene in the adenovirus genome, e.g. using the Ad5 genome as a reference. It is preferred that the polynucleotide also comprises sequences required for packaging of the polynucleotide into an adenoviral particle.

Adenovirus PanAd1

In a preferred embodiment of the first aspect of the invention, the isolated polynucleotide encodes an adenoviral fiber protein with an amino acid sequence according to SEQ ID NO: 19 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has an amino acid sequence which is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or at least 99%, more preferably at least 85% and most preferable at least 99% identical over its entire length to the amino acid sequence of SEQ ID NO: 19.

In a preferred embodiment of the second aspect of the invention, the isolated polynucleotide encodes an adenoviral hexon protein with an amino acid sequence according to SEQ ID NO: 25 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has an amino acid sequence which is at least 95%, 98%, 99%, 99.5%, 99.9% or at least 99.95%, more preferably at least 98% and most preferably at least 99% identical over its entire length to the amino acid sequence of SEQ ID NO: 25.

In a preferred embodiment of the third aspect of the invention, the isolated polynucleotide encodes an adenoviral penton protein with an amino acid sequence according to SEQ ID NO: 31 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has an amino acid sequence which is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or at least 99%, more preferably at least 85% and most preferable at least 90% identical over its entire length to the amino acid sequence of SEQ ID NO: 31.

In a most preferred embodiment, the polynucleotide of the invention consists of or comprises a polynucleotide which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% identical and most preferably at least 99% or 100% identical over its entire length to a sequence that consists of SEQ ID NO: 13 or to a sequence that consists of SEQ ID NO: 13 but lacks any of the genomic regions E1A, E1B, E2A, E2B, E3 and/or E4 of SEQ ID NO: 13, most preferably that lacks the genomic regions E1, E3 and E4 of SEQ ID NO: 13.

In a further aspect the invention relates to a polynucleotide comprising the first, the second, the third, the first and second, the first and third, the second and third or the first, second and third aspect. It is preferred that the polynucleotide comprising this or these polynucleotide(s) comprises other adenoviral genes and nucleotide segments, which are adjacent to the hexon,

penton and/or fiber gene in the adenovirus genome, e.g. using the PanAd1 genome as set out in SEQ ID NO: 13. It is preferred that the polynucleotide also comprises sequences required for packaging of the polynucleotide into an adenoviral particle.

Adenovirus PanAd2

In a preferred embodiment of the first aspect of the invention, the isolated polynucleotide encodes an adenoviral fiber protein with an amino acid sequence according to SEQ ID NO: 50 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has an amino acid sequence which is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or at least 99%, more preferably at least 85% and most preferable at least 99% identical over its entire length to the amino acid sequence of SEQ ID NO: 50.

In a preferred embodiment of the second aspect of the invention, the isolated polynucleotide encodes an adenoviral hexon protein with an amino acid sequence according to SEQ ID NO: 51 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has an amino acid sequence which is at least 95%, 98%, 99%, 99.5%, 99.9% or at least 99.95%, more preferably at least 98% and most preferably at least 99% identical over its entire length to the amino acid sequence of SEQ ID NO: 51.

In a preferred embodiment of the third aspect of the invention, the isolated polynucleotide encodes an adenoviral penton protein with an amino acid sequence according to SEQ ID NO: 52 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has an amino acid sequence which is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or at least 99%, more preferably at least 85% and most preferable at least 90% identical over its entire length to the amino acid sequence of SEQ ID NO: 52.

In a most preferred embodiment, the polynucleotide of the invention consists of or comprises a polynucleotide which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% identical and most preferably at least 99% or 100% identical over its entire length to a sequence that consists of SEQ ID NO: 62 or to a sequence that consists of SEQ ID NO: 62 but lacks any of the genomic regions E1A, E1B, E2A, E2B, E3 and/or E4 of SEQ ID NO: 62, most preferably that lacks the genomic regions E1, E3 and E4 of SEQ ID NO: 62.

In a further aspect the invention relates to a polynucleotide comprising the first, the second, the third, the first and second, the first and third, the second and third or the first, second and third aspect. It is preferred that the polynucleotide comprising this or these polynucleotide(s) comprises other adenoviral genes and nucleotide segments, which are adjacent to the hexon, penton and/or fiber gene in the adenovirus genome, e.g. using the PanAd1 genome as set out in SEQ ID NO: 62. It is preferred that the polynucleotide also comprises sequences required for packaging of the polynucleotide into an adenoviral particle.

Adenovirus PanAd3

In a preferred embodiment of the first aspect of the invention, the isolated polynucleotide encodes an adenoviral fiber protein with an amino acid sequence according to SEQ ID NO: 53 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has an amino acid sequence which is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or at least 99%, more preferably at least 85% and most preferable at least 99% identical over its entire length to the amino acid sequence of SEQ ID NO: 53.

In a preferred embodiment of the second aspect of the invention, the isolated polynucleotide encodes an adenoviral hexon protein with an amino acid sequence according to SEQ ID NO: 54 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has an amino acid sequence which is at least 95%, 98%, 99%, 99.5%, 99.9% or at least 99.95%, more preferably at least 98% and most preferably at least 99% identical over its entire length to the amino acid sequence of SEQ ID NO: 54.

In a preferred embodiment of the third aspect of the invention, the isolated polynucleotide encodes an adenoviral penton protein with an amino acid sequence according to SEQ ID NO: 55 or a functional derivative thereof, wherein the functional derivative (i) does not comprise more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 10 deleted, inserted, modified and/or substituted amino acids or (ii) has an amino acid sequence which is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or at least 99%, more preferably at least 85% and most preferable at least 90% identical over its entire length to the amino acid sequence of SEQ ID NO: 55.

In a most preferred embodiment, the polynucleotide of the invention consists of or comprises a polynucleotide which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%

identical and most preferably at least 99% or 100% identical over its entire length to a sequence that consists of SEQ ID NO: 63 or to a sequence that consists of SEQ ID NO: 63 but lacks any of the genomic regions E1A, E1B, E2A, E2B, E3 and/or E4 of SEQ ID NO: 63, most preferably that lacks the genomic regions E1, E3 and E4 of SEQ ID NO: 63.

In a further aspect the invention relates to a polynucleotide comprising the first, the second, the third, the first and second, the first and third, the second and third or the first, second and third aspect and most preferably the first, second and third aspect of the invention. It is preferred that the polynucleotide comprising this or these polynucleotide(s) comprises other adenoviral genes and nucleotide segments, which are adjacent to the hexon, penton and/or fiber gene in the adenovirus genome, e.g. using the PanAd1 genome as set out in SEQ ID NO: 63. It is preferred that the polynucleotide also comprises sequences required for packaging of the polynucleotide into an adenoviral particle.

In a recombinant adenovirus, a fiber, hexon and penton protein according to the first, second and third aspect of the invention, and according to the respective preferred embodiments disclosed herein, contributes each individually to reduce the interaction of said recombinant adenovirus with human and/or rodent neutralizing antibodies. Accordingly, polynucleotides which encode said fiber, hexon and/or penton protein of the present invention are useful to construct enhanced recombinant adenoviruses. Thus, in a further, fourth aspect the invention provides a polynucleotide comprising at least one, preferably at least two and most preferably three isolated polynucleotides selected from the group of polynucleotides consisting of a polynucleotide according to the first aspect of the invention, the second aspect of the invention and the third aspect of the invention. Thus, most preferably, the fourth aspect is an isolated polynucleotide comprising the first, second and third aspect of the invention. In a preferred embodiment, the polynucleotide according to the fourth aspect of the invention is a polynucleotide selected from the group consisting of:

(i) a polynucleotide comprising one polynucleotide according to the first, second or third aspect of the invention;

(ii) a polynucleotide comprising a polynucleotide according to the first aspect of the invention and a polynucleotide according to the second aspect of the invention;

(iii) a polynucleotide comprising a polynucleotide according to the first aspect of the invention and a polynucleotide according to the third aspect of the invention;

(iv) a polynucleotide comprising a polynucleotide according to the second aspect of the invention and a polynucleotide according to the third aspect of the invention; and

(v) a polynucleotide comprising a polynucleotide according to the first, second and third aspect of the invention;

wherein it is preferred that said polynucleotides comprised in the polynucleotide according to (i) through (v) are selected from the *same* adenovirus isolate, e.g. all three polynucleotides encoding fiber, hexon and penton protein or functional derivative thereof, respectively, are from only one of the following adenoviruses: ChAd55, ChAd73, ChAd83, ChAd146, ChAd147 PanAd1, PanAd2 or PanAd3. Furthermore, it is preferred that in the fourth aspect of the invention or in a preferred embodiment thereof, e.g. as outlined above, each "functional derivative" does not comprise more than 10, more than 5 or more than 3 amino acid changes (i.e. deleted, inserted, modified and/or substituted amino acids).

Table 2 below lists a number of particularly preferred embodiments of the polynucleotide of the fourth aspect of invention outlined above. Preferred is a polynucleotide selected from polynucleotides A1 through AF1 shown in Table 2, wherein the polynucleotide comprises three polynucleotides according to alternative (c) of the first, second and third aspect of the invention, each of which respectively encodes an adenoviral fiber, hexon and penton protein or a functional derivative thereof. Table 2 below shows the minimal sequence identity (i.e. at least the indicated sequence identity) which each of said three encoded proteins has to have over its entire length to the amino acid sequence according to the SEQ ID NO which is also shown in Table 2:

Table 2

	Fiber Protein		Hexon Protein		Penton Protein	
Preferred embodiment	Minimal %-Identity	to SEQ ID NO:	Minimal %-Identity	to SEQ ID NO:	Minimal %-Identity	to SEQ ID NO:
A1 – ChAd55	85%	14	95%	20	98%	26
B1 – ChAd73	98%	15	95%	21	98%	27
C1 – ChAd83	100%	16	95%	22	98%	28
D1 – ChAd146	100%	17	95%	23	98%	29
E1 – ChAd147	85%	18	95%	24	98%	30
F1 – PanAd1	85%	19	95%	25	98%	31
G1 – ChAd55	90%	14	95%	20	100%	26
H1 – ChAd73	90%	15	95%	21	98%	27
I1 – ChAd83	90%	16	95%	22	98%	28
J1 – ChAd146	90%	17	95%	23	98%	29
K1 – ChAd147	90%	18	95%	24	98%	30
L1 – PanAd1	90%	19	95%	25	90%	31
M1 – ChAd55	98%	14	98%	20	98%	26
N1 – ChAd73	98%	15	98%	21	98%	27
O1 – ChAd83	98%	16	98%	22	98%	28
P1 – ChAd146	98%	17	98%	23	98%	29
Q1 – ChAd147	98%	18	98%	24	98%	30
R1 – PanAd1	98%	19	98%	25	98%	31
S1 – ChAd55	99%	14	99%	20	99%	26
T1 – ChAd73	99%	15	99%	21	99%	27
U1 – ChAd83	99%	16	99%	22	99%	28
V1 – ChAd146	99%	17	99%	23	99%	29

W1 – ChAd147	99%	18	99%	24	99%	30
X1 – PanAd1	99%	19	99%	25	99%	31
Y1 – PanAd2	80%	50	95%	51	85%	52
Z1 – PanAd2	90%	50	95%	51	90%	52
AA1 – PanAd2	98%	50	98%	51	98%	52
AB1 – PanAd2	99%	50	99%	51	99%	52
AC1 – PanAd3	75%	53	95%	54	85%	55
AD1 – PanAd3	90%	53	95%	54	90%	55
AE1 – PanAd3	98%	53	98%	54	98%	55
AF1 – PanAd3	99%	53	99%	54	99%	55

For example, preferred polynucleotide A1 as shown in Table 1 above comprises:

(i) a polynucleotide encoding a polypeptide having an amino acid sequence which is at least 85% identical over its entire length to SEQ ID NO: 14;

(ii) a polynucleotide encoding a polypeptide having an amino acid sequence which is at least 95% identical over its entire length to SEQ ID NO: 20; and

(iii) a polynucleotide encoding a polypeptide having an amino acid sequence which is at least 98% identical over its entire length to SEQ ID NO: 26;

As mentioned above it is most preferred that said "functional derivative" of a polynucleotide listen in table 2 does not comprise more than 10 amino acid changes (i.e. deleted, inserted, modified and/or substituted amino acids).

Table 3 below lists further preferred embodiments of the polynucleotide of the fourth aspect of the invention. Preferred is a polynucleotide selected from polynucleotides A2 through J2 selected from Table 3, wherein the polynucleotide comprises three polynucleotides designated, "Polynucleotide 1", "Polynucleotide 2" and "Polynucleotide 3", wherein each respective polynucleotide has at least the indicated sequence identity over its entire length to the corresponding polynucleotide according to the SEQ ID NO shown in Table 3:

Table 3

	Polynucleotide 1		Polynucleotide 2		Polynucleotide 3	
Preferred embodiment	Minimal %-Identity	to SEQ ID NO: (polynucleotide encoding Fiber protein)	Minimal %-Identity	to SEQ ID NO: (polynucleotide encoding Hexon protein)	Minimal %-Identity	to SEQ ID NO: (polynucleotide encoding Penton protein)
A2 – ChAd55	98%	32	98%	38	98%	44
B2 – ChAd73	98%	33	98%	39	98%	45
C2 – ChAd83	98%	34	98%	40	98%	46
D2 – ChAd146	98%	35	98%	41	98%	47
E2 – ChAd147	98%	36	98%	42	98%	48

F2 – PanAd1	98%	37	98%	43	98%	49
G2 – ChAd55	99%	32	99%	38	99%	44
H2 – ChAd73	99%	33	99%	39	99%	45
I2 – ChAd83	99%	34	99%	40	99%	46
J2 – ChAd146	99%	35	99%	41	99%	47
K2 – ChAd147	99%	36	99%	42	99%	48
L2 – PanAd1	99%	37	99%	43	99%	49
G2 – PanAd2	98%	56	98%	57	98%	58
H2 – PanAd2	99%	56	99%	57	99%	58
I2 – PanAd3	98%	59	98%	60	98%	61
J2 – PanAd3	99%	59	99%	60	99%	61

Thus, as an example, preferred embodiment A2 ("A2 – ChAd55") of Table 3 above is a polynucleotide comprising:

(i) a polynucleotide that is at least 98% identical to SEQ ID NO: 32 over its entire length;

(ii) a polynucleotide that is at least 98% identical to SEQ ID NO: 38 over its entire length; and

(iii) a polynucleotide that is at least 98% identical to SEQ ID NO: 44 over its entire length.

Table 4 below lists a number of further particularly preferred embodiments of the polynucleotide of the fourth aspect of invention outlined above. Preferred is a polynucleotide selected from polynucleotides A3 through H3 shown in Table 4, wherein the polynucleotide encodes an adenoviral fiber, hexon and penton protein according to the indicated SEQ ID NO or a functional derivative thereof, wherein all three proteins and/or encoded functional derivatives in total comprises equal or less than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more than 100, preferably not more than 20 deleted, inserted, modified and/or substituted amino acids:

Table 4

	Fiber Protein	Hexon Protein	Penton Protein
Preferred embodiment	according to SEQ ID NO:	according to SEQ ID NO:	according to SEQ ID NO:
A3 – ChAd55	14	20	26
B3 – ChAd73	15	21	27
C3 – ChAd83	16	22	28
D3 – ChAd146	17	23	29
E3 – ChAd147	18	24	30
F3 – PanAd1	19	25	31
G3 – PanAd2	50	51	52
H3 – PanAd3	53	54	55

In another embodiment of the polynucleotide of the fourth aspect of invention, the polynucleotide encodes an adenoviral fiber and hexon protein of the same strain according to the respective SEQ ID NO as shown in Table 4 or functional derivatives thereof. In a further embodiment of the polynucleotide of the fourth aspect of invention, the polynucleotide encodes

an adenoviral fiber and penton protein of the same strain according to the respective SEQ ID NO as shown in Table 4 or functional derivatives thereof. In a further embodiment of the polynucleotide of the fourth aspect of invention, the polynucleotide encodes an adenoviral hexon and penton protein of the same strain according to the respective SEQ ID NO as shown in Table 4 or functional derivatives thereof. In this context, said functional derivative comprises in each instance less than 1, 2, 3, 4, 5, 6, 7, 8, 9 or less than 10, most preferably less than 3 deleted, inserted, modified and/or substituted amino acids.

In a further preferred embodiment of the fourth aspect of the invention, the polynucleotide consists of or comprises a polynucleotide which is at least 90%, 91%, 92%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9% or 100%, preferably 98% identical over its entire length to a sequence that (i) consists of any one of SEQ ID NO: 13, 62, 63 or 65 or to (ii) a sequence that consists of any one of SEQ ID NO: 13, 62 63 or 65 that lacks one or more of the genomic regions E1A, E1B, E2A, E2B, E3 ORF1, E3 ORF2, E3 ORF3, E3 ORF4, E3 ORF5, E3 ORF6, E3 ORF7, E3 ORF8, E3 ORF9, E4 ORF7, E4 ORF6, E4 ORF5, E4 ORF4, E4 ORF3, E4 ORF2 and/or E4 ORF1. Thus, the aforementioned one or more genomic regions will preferably not be considered in the alignment when determining the percent identity. In another preferred embodiment of the isolated polynucleotide of the invention, the polynucleotide comprises or consists of SEQ ID NO: 13, 62, 63 or 65, wherein one or more of the genomic regions E1A, E1B, E2A, E2B, E3 ORF1, E3 ORF2, E3 ORF3, E3 ORF4, E3 ORF5, E3 ORF6, E3 ORF7, E3 ORF8, E3 ORF9, E4 ORF7, E4 ORF6, E4 ORF5, E4 ORF4, E4 ORF3, E4 ORF2 and E4 ORF1 are deleted from SEQ ID NO: 13, 62, 63 or 65, respectively, or substituted with a transgene or an expression cassette encoding a heterologous protein as described herein. In a most preferred embodiment adenoviral regions E1, E3 and/or E4 are deleted as also exemplified in example 2. The aforementioned preferred polynucleotides, which lack one or more of the indicated genomic regions may further comprise a polynucleotide sequence encoding for a heterologous protein or an expression cassette comprising such a polynucleotide sequence encoding for a heterologous protein. Said polynucleotide sequence encoding for a heterologous protein and said expression cassette comprising such a polynucleotide sequence encoding for a heterologous protein may be inserted into e.g. the deleted regions of the polynucleotide of the invention as is well known in the art and also described in the examples below. Said heterologous protein may be a molecule for delivery into a target cell such as described herein, e.g. a polynucleotide encoding an antigenic protein or a fragment thereof, preferably an antigenic protein or a fragment of a pathogen such as HIV gag protein, a tumour antigen or a protein of the herpes simplex virus as described in the examples. Thus, in a preferred embodiment, the isolated polynucleotide according to the invention further comprises a polynucleotide encoding an antigen selected from the group consisting of a virus

antigen, an antigen of a pathogenic bacterium and a tumorantigen. In one embodiment, said heterologous protein can thus be an antigen selected from the group consisting of an RNA virus antigen, an antigen of a pathogenic bacterium and a tumorantigen. An antigen refers to any protein or peptide capable of eliciting an immune response in a mammal. An antigen comprises preferably at least 8 amino acids and most preferably comprises between 8 and 12 amino acids. Thus, when determining the sequence identity, the genomic regions E1A, E1B, E2A, E2B, E3 and/or E4 are preferably not considered in the alignment, i.e. the alignment is done using a sequence that consists of the entire sequence SEQ ID NO: 13, 62 63 or 65 but excluding the genomic regions E1A, E1B, E2A, E2B, E3, E4 and/or any polynucleotide encoding a heterologous polypeptide or expression cassette comprising such polynucleotide. As also mentioned above, it is preferred that the polynucleotide according to the fourth aspect of the invention and all its preferred embodiments encodes functional hexon, penton and/or fiber capsid proteins or functional derivatives thereof, e.g. the encoded proteins have the same function as the respective capsid proteins or fragments thereof in an *infectious* adenovirus virion. Thus, a recombinant adenovirus comprising in its capsid said encoded recombinant penton, hexon and/or fiber proteins or functional derivatives thereof is capable of entering a host cell. It is further preferred that the capsid proteins or functional derivatives thereof according to the invention or encoded by polynucleotides of the invention have no seroprevalence in human.

The invention further provides an isolated protein encoded by the isolated polynucleotide according to the invention, i.e. an isolated adenoviral capsid polypeptide encoded by the isolated polynucleotide according to the first, second and/or third aspect of the invention or a functional derivative thereof. In this context, the "functional derivative" in one embodiment does not comprise more than 5, 10 or not more than 25 amino acid changes (i.e. deleted, inserted, modified and/or substituted amino acids).

The invention further relates to a vector comprising an isolated polynucleotide according to the invention.

Preferably, the vector does not comprise a gene in a genomic region selected from the group of genomic regions consisting of E1A, E1B, E2A, E2B, E3 and E4, and/or comprises at least one gene of a genomic region selected from the group of E1A, E1B, E2A, E2B, E3 and E4, wherein said at least one gene comprises a deletion and/or mutation which renders the at least one gene non-functional. One possibility to render one of these gene products non-functional is to introduce one or more artificial stop-codons (e.g. TAA) into the open reading frame of these genes. Methods of rendering the virus replication-defective are well known in the art (see e.g. Brody et al, 1994 Ann NY Acad Sci., 716: 90-101).

In some embodiments the polynucleotide of the invention comprises a polynucleotide encoding a hexon protein; penton protein; fiber protein; hexon protein and penton protein; hexon protein and fibre protein; penton protein and fibre protein; or hexon protein, penton protein and fibre protein of the invention and further comprises additional adenoviral polynucleotides. Thus, in one preferred embodiment, the isolated polynucleotide according to the invention comprises at least one of the following:

(a) an adenoviral 5'-inverted terminal repeat (ITR);

(b) an adenoviral Ela region, or a fragment thereof selected from among the 13S, 12S and 9S regions;

(c) an adenoviral Elb region, or a fragment thereof selected from among the group consisting of the small T, large T and IX regions;

(d) an adenoviral E2b region; or a fragment thereof selected from among the group consisting of the small pTP, Polymerase and IVa2 regions;

(e) an adenoviral L1 region, or a fragment thereof, said fragment encoding an adenoviral protein selected from the group consisting of the 28.1 kD protein, polymerase, agnoprotein, 52/55 kDa protein, and IIIa protein;

(f) an adenoviral L2 region or a L2 region comprising a polynucleotide encoding the penton protein of the invention, or a fragment thereof, said fragment encoding an adenoviral protein selected from the group consisting of a penton protein or the penton protein of the invention, VII, V, and Mu protein;

(g) an adenoviral L3 region or a L3 region comprising a polynucleotide encoding the hexon protein of the invention, or a fragment thereof, said fragment encoding an adenoviral protein selected from the group consisting of the VI protein, hexon protein or the hexon protein of the invention and endoprotease;

(h) an adenoviral E2a region;

(i) an adenoviral L4 region, or a fragment thereof said fragment encoding an adenoviral protein selected from the group consisting of the 100 kD protein, the 33 kD homolog, and protein VIII;

(j) an adenoviral E3 region, or a fragment thereof selected from the group consisting of E3 ORF1, E3 ORF2, E3 ORF3, E3 ORF4, E3 ORF5, E3 ORF6, E3 ORF7, E3 ORF8, and E3 ORF9;

(k) an adenoviral L5 region or a L5 region comprising a polynucleotide encoding the fibre protein of the invention, or a fragment thereof said fragment encoding the fiber protein or the fiber protein of the invention;

(l) an adenoviral E4 region, or a fragment thereof selected from the group consisting of E4 ORF7, E4 ORF6, E4 ORF5, E4 ORF4, E4 ORF3, E4 ORF2, and E4 ORF1; in particular ORF6 of said E4 region;

and /or

(m) an adenoviral 3'-ITR.

In some embodiments of the aforementioned polynucleotide it may be desirable as also described above that preferably, the polynucleotide does not comprise an ORF of a genomic region as outlined above (such as e.g. region E3 and/or E4 as defined in example 2) and/or comprises an adenoviral gene which comprises a deletion and/or mutation which renders the at least one gene non-functional. In these preferred embodiments the suitable adenoviral regions will be modified to not include the aforementioned gene(s) or to render the selected gene(s) non-functional. Any adenoviral gene deletions will make space to insert transgenes such as a minigene cassette as described herein. Furthermore, gene deletions can be used to generate adenoviral vectors which are incapable to replicate without the use of a packaging cell line or a helper virus as is well known in the art. Thus, the final recombinant adenovirus comprising a polynucleotide as outlined above which comprises one or more of the specified gene / region deletions or loss-of-function mutations can provide a safer recombinant adenovirus for e.g. gene therapy or vaccination.

In a particularly preferred embodiment, the polynucleotide of the invention comprises at least one of the following:

(a) the 5'-inverted terminal repeat (ITR) region of any one of SEQ ID NO: 13, 62, 63 or 65;

(b) the adenovirus Ela region of any one of SEQ ID NO: 13, 62, 63 or 65, or a fragment thereof selected from among the 13S, 12S and 9S regions;

(c) the adenovirus Elb region of any one of SEQ ID NO: 13, 62, 63 or 65, or a fragment thereof selected from among the group consisting of the small T, large T and IX regions;

(d) the adenovirus E2b region of any one of SEQ ID NO: 13, 62, 63 or 65; or a fragment thereof selected from among the group consisting of the small pTP, Polymerase and IVa2 regions;

(e) the adenovirus L1 region of any one of SEQ ID NO: 13, 62, 63 or 65, or a fragment thereof, said fragment encoding an adenoviral protein selected from the group consisting of the 28.1 kD protein, polymerase, agnoprotein, 52/55 kDa protein, and IIIa protein;

(f) the adenovirus L2 region of any one of SEQ ID NO: 13, 62, 63 or 65, or a fragment thereof, said fragment encoding an adenoviral protein selected from the group consisting of the penton protein with the amino acid sequence of SEQ ID NO: 31, 52 or 55, VII, V, and Mu protein;

(g) the adenovirus L3 region of any one of SEQ ID NO: 13, 62, 63 or 65, or a fragment thereof, said fragment encoding an adenoviral protein selected from the group consisting of the VI protein, hexon protein with the amino acid sequence of SEQ ID NO: 25, 51 or 54 and endoprotease;

(h) the adenovirus E2a region of any one of SEQ ID NO: 13, 62, 63 or 65;

(i) the adenovirus L4 region of any one of SEQ ID NO: 13, 62, 63 or 65, or a fragment thereof said fragment encoding an adenoviral protein selected from the group consisting of the 100 kD protein, the 33 kD homolog, and protein VIII;

(j) the adenovirus E3 region of any one of SEQ ID NO: 13, 62, 63 or 65, or a fragment thereof selected from the group consisting of E3 ORF1, E3 ORF2, E3 ORF3, E3 ORF4, E3 ORF5, E3 ORF6, E3 ORF7, E3 ORF8, and E3 ORF9;

(k) the adenovirus L5 region of any one of SEQ ID NO: 13, 62, 63 or 65, or a fragment thereof said fragment encoding the fiber protein with the amino acid sequence of SEQ ID NO:19, 50 or 53;

(l) the adenovirus E4 region of any one of SEQ ID NO: 13, 62, 63 or 65, or a fragment thereof selected from the group consisting of E4 ORF7, E4 ORF6, E4 ORF5, E4 ORF4, E4 ORF3, E4 ORF2, and E4 ORF1; or ORF6 of Ad5 E4 region (SEQ ID NO: 64); and

(m) the 3'-ITR of any one of SEQ ID NO: 13, 62, 63 or 65.

In one embodiment the isolated polynucleotide of the invention further encodes one or more, preferably all of the following adenoviral proteins: protein VI, protein VIII, protein IX, protein IIIa and protein IVa2. Preferably these proteins are encoded by from the respective open reading frames of the PanAd1, PanAd2 or PanAd3 genomic sequence disclosed herein. An average person skilled in the art of recombinant adenoviruses is well aware of how to determine the open reading frames that encode for the above specified adenoviral proteins. He is also aware of the structure of adenoviral genomes and can map, without undue burden, the individual adenoviral regions and ORFs outlined herein to e.g. any of the novel adenoviral genomes PanAd1, PanAd2 or PanAd3 of the invention.

In order to express a polynucleotide, preferably a cDNA, encoding one or more adenoviral proteins of the invention, one can subclone said polynucleotide into an expression vector that contains a strong promoter to direct transcription, a transcription/translation terminator, and a ribosome-binding site for translational initiation. Suitable bacterial promoters are well known in the art, e.g., E. coli, Bacillus sp., and Salmonella, and kits for such expression systems are commercially available. Similarly eukaryotic expression systems for mammalian cells, yeast, and insect cells are well known in the art and are also commercially available.

In addition to the promoter, the expression vector typically contains a transcription unit or expression cassette that contains all the additional elements required for the expression of the adenoviral protein-encoding nucleic acid in host cells. A typical expression cassette thus contains a promoter operatively linked to the nucleic acid sequence encoding the adenoviral protein/polypeptide and signals required for efficient polyadenylation of the transcript, ribosome binding sites, and translation termination. Additional elements of the cassette may include, for example enhancers. An expression cassette should also contain a transcription termination region downstream of the structural gene to provide for efficient termination. The termination region may be obtained from the same gene as the promoter sequence or may be obtained from different genes.

The particular expression vector used to transport the genetic information into the cell is not particularly critical. Any of the conventional vectors used for expression in eukaryotic or prokaryotic cells may be used. Standard bacterial expression vectors include plasmids such as pBR322 based plasmids, pSKF, pET23D, and fusion expression systems such as GST and LacZ, but there are many more known in the art to the skilled person that can be usefully employed.

Expression vectors containing regulatory elements from eukaryotic viruses are typically used in eukaryotic expression vectors, e.g. SV40 vectors, papilloma virus vectors, and vectors derived from Epstein-Barr virus. Other exemplary eukaryotic vectors include pMSG, pAV009/A.sup.+, pMTO10/A.sup.+, pMAMneo-5, baculovirus pDSVE, pcDNA3.1, pIRES and any other vector allowing expression of proteins under the direction of e.g. the HCMV immediate-early promoter, SV40 early promoter, SV40 late promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or other promoters shown effective for expression in eukaryotic cells.

Some expression systems have markers that provide gene amplification such as thymidine kinase, hygromycin B phosphotransferase, and dihydrofolate reductase. Alternatively, high yield expression systems not involving gene amplification are also suitable.

The elements that may also be included in expression vectors include a replicon that functions in E. coli, a gene encoding drug resistance to permit selection of bacteria that harbor recombinant plasmids, and unique restriction sites in nonessential regions of the plasmid to allow insertion of eukaryotic sequences. The particular drug resistance gene chosen is not critical - any of the many drug resistance genes known in the art are suitable. The prokaryotic sequences are optionally chosen such that they do not interfere with the replication of the DNA in eukaryotic cells, if necessary.

Standard transfection methods can be used to produce bacterial, mammalian, yeast or insect cell lines. Any of the well-known procedures for introducing foreign polynucleotide

sequences into host cells may be used. For example, commercially available liposome-based transfection kits such as Lipofectamine™ (Invitrogen), commercially available lipid-based transfection kits such as Fugene (Roche Diagnostics), polyethylene glycol-based transfection, calcium phosphate precipitation, gene gun (biolistic), electroporation, or viral infection and any of the other well known methods for introducing cloned genomic DNA, cDNA, synthetic DNA or other foreign genetic material into a host cell may be used. It is only necessary that the particular genetic engineering procedure used be capable of successfully introducing at least one gene into the host cell capable of expressing the receptor.

An expressed adenoviral protein can be optionally purified using standard techniques. For example, the cells may be lysed either mechanically or by osmotic shock before being subject to precipitation and chromatography steps, the nature and sequence of which will depend on the particular recombinant material to be recovered. Alternatively, the recombinant protein may be secreted and recovered from the culture medium in which the recombinant cells had been cultured as is known in the art of protein expression.

In one preferred embodiment the vector of the invention is a plasmid vector, e.g. an expression vector. A plasmid vector according to the invention can also be used to generate a recombinant adenovirus.

Thus, a further aspect of the present invention is a recombinant adenovirus, preferably a replication-incompetent adenovirus, comprising an isolated polynucleotide according to the invention and/or at least one isolated adenoviral capsid polypeptide according to the invention. Preferably the recombinant adenovirus of the invention comprises a hexon a fiber and a penton protein of the present invention, e.g. a combination as outlined in Table 2 above. In a preferred embodiment, the recombinant adenovirus is characterized in that it is capable of infecting a human cell - preferably capable of infecting a human cell after said adenovirus was incubated for one hour in a human blood serum derived from a human that has not previously been exposed to a chimpanzee adenovirus.

As the sequence information of the novel hexon, penton and fiber proteins of the invention are provided, said recombinant adenovirus is obtainable e.g. by constructing a recombinant adenovirus which is composed of the usual adenoviral proteins but which has a capsid that comprises at least one isolated adenoviral capsid polypeptide according to the invention or a functional derivative thereof. In this regard it is preferred that the recombinant adenovirus comprises an L2 region which comprises a polynucleotide sequence encoding the penton protein of the invention, an L3 region which comprises a polynucleotide sequence encoding the hexon protein of the invention and/or an L5 region which comprises a polynucleotide sequence encoding the fiber protein of the invention. Most preferably said

recombinant adenovirus comprises an L2 region, an L3 region and an L5 region encoding, respectively, at least for the penton, hexon and fiber protein of the invention.

Methods for the construction of recombinant adenoviruses are well known in the art. Useful techniques for the preparation of recombinant adenoviruses are, for example, reviewed in Graham & Prevec, 1991 In Methods in Molecular Biology: Gene Transfer and Expression Protocols, (Ed. Murray, EJ.), p. 109; and Hitt et al., 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" Advances in Pharmacology 40:137-206. Further methods are described in WO 2006/086284. For the preparation of replication deficient adenoviruses, one or several of the E1A, E1B, E2A, E2B, E3 and E4 gene products may be expressed in a complementing cell line that can be used for the propagation and rescue of recombinant adenoviruses that are replication-incompetent, because they lack e.g. one of the aforementioned gene products. The use of such cell-lines is also described in the references outlined above.

In one embodiment, the polynucleotides of the invention (or vectors comprising said polynucleotides of the invention as described herein) are used to produce recombinant adenoviral particles. The recombinant adenoviruses are preferably functionally deleted as mentioned above in one or more adenoviral regions such as e.g. the Ela or Elb regions, and optionally bearing other mutations, e. g., temperature-sensitive mutations or deletions in other adenoviral genes. In other embodiments, it is desirable to retain an intact Ela and/or Elb region in the recombinant adenoviruses. Such an intact El region may be located in its native location in the adenoviral genome or placed in the site of a deletion in the native adenoviral genome (e.g., in the E3 region).

In the construction of adenovirus vectors for delivery of a gene to a host, e.g. human (or other mammalian) cell, a range of adenovirus nucleic acid sequences can be employed in the vectors of the invention. For example, all or a portion of the adenovirus delayed early gene E3 may be eliminated from the adenovirus sequence which forms a part of the recombinant virus. The function of simian E3 is believed to be irrelevant to the function and production of the recombinant virus particle. In some embodiments, adenovirus vectors may also be constructed having a deletion of at least the ORF6 region of the E4 gene, and more desirably because of the redundancy in the function of this region, the entire E4 region. Still another vector of this invention contains a deletion in the delayed early gene E2a. Deletions may also be made in any of the late genes L1 through L5 of the simian adenovirus genome. Similarly, deletions in the intermediate genes IX and IVa2 may be useful for some purposes. Other deletions may be made in the other structural or non-structural adenovirus genes. The above discussed deletions may be used individually, i. e., an adenovirus sequence for use in the present invention may contain deletions in only a single region. Alternatively, deletions of entire genes or portions thereof

effective to destroy their biological activity may be used in any combination. For example, in one exemplary vector according to the invention, the adenovirus sequence may have deletions of the El and the E4 region, or of the El, E2a and E3 region, or of the E1 and E3 regions, or of El, E2a and E4 regions, with or without deletion of E3, and so on. As discussed above, such deletions may be used in combination with other adenoviral gene mutations, such as temperature-sensitive mutations, to achieve a desired result.

An adenoviral vector lacking any essential adenoviral sequences (e. g., a region selected from Ela, Elb, E2a, E2b, E4 ORF6, L1 or L4) may be cultured in the presence of the missing adenoviral gene products which are required for viral infectivity and propagation of an adenoviral particle. These helper functions may be provided by culturing the adenoviral vector in the presence of one or more helper constructs (e. g. , a plasmid or virus) or a packaging host cell (complementing cell line as also described above). See, for example, the examples included herein and the techniques described for preparation of a "minimal" human adenovirus vector in International Patent Application WO96/13597 published May 9, 1996, and incorporated herein by reference.

Useful helper viruses contain selected adenovirus gene sequences that complement the respective genes that are deleted in preferred embodiments of the adenovirus vector of the invention and/or that are not expressed by the packaging cell line in which the vector is transfected. In one embodiment, the helper virus is replication-defective and contains a variety of adenovirus genes in addition to the sequences described above.

Helper viruses may also be formed into poly-cation conjugates as described in Wu et al, J. Biol. Chem., 264: 16985-16987 (1989); K. J. Fisher and J. M. Wilson, Biochem. J., 299: 49 (April 1, 1994). A helper virus may optionally contain a second reporter minigene. A number of such reporter genes are known to the art. The presence of a reporter gene on the helper virus which is different from the transgene on the adenovirus vector allows both the Ad vector and the helper virus to be independently monitored. This second reporter may be used to facilitate separation between the resulting recombinant virus and the helper virus upon purification.

To generate recombinant adenoviruses (Ad) deleted in any of the genes described in the context of preferred embodiments herein, the function of the deleted gene region, if essential to the replication and infectivity of the virus, is preferably supplied to the recombinant virus by a helper virus or cell line, i. e. , a complementation or packaging cell line. In many circumstances, a cell line expressing the human E1 can be used to transcomplement the vector used to generate recombinant adenoviruses. This is particularly advantageous because, due to the diversity between the polynucleotide sequences of the invention and the human adenoviral E1 sequences found in currently available packaging cells, the use of the current human E1-containing cells

will prevent the generation of replication-competent adenoviruses during the replication and production process. However, in certain circumstances, it will be desirable to utilize a cell line which expresses the E1 gene products for the production of an E1-deleted recombinant adenovirus.

If desired, one may utilize the sequences provided herein to generate a packaging cell or cell line that expresses, at a minimum, the adenovirus E1 gene from a ChAd55, ChAd73, ChAd83, ChAd146, ChAd147, PanAd1, PanAd2 or PanAd3 adenovirus under the transcriptional control of a promoter for expression in a selected parent cell line, such as e.g. a HeLa cell. Inducible or constitutive promoters may be employed for this purpose. Examples of promoters are provided e.g. in the examples described herein. Such E1-expressing cell lines are useful in the generation of recombinant adenovirus E1 deleted vectors. Additionally, or alternatively, the invention provides cell lines that express one or more adenoviral gene products, e. g., Ela, Elb, E2a, and/or E4 ORF6, preferably Ad5 E4 ORF6 (see also the examples below), which can be constructed using essentially the same procedures for use in the generation of recombinant adenoviral vectors. Such cell lines can be utilized to transcomplement adenovirus vectors deleted in essential genes that encode those products, or to provide helper functions necessary for packaging of a helper- dependent virus (e. g., adeno-associated virus).

Generally, when delivering a vector of the invention comprising e.g. a minigene by transfection, the vector is delivered in an amount from about 0.1 μg to about 100 μg DNA, and preferably about 10 to about 50 μg DNA to about 1 x 10^4 cells to about 1 x 10^3 cells, and preferably about 10^5 cells. However, the relative amounts of vector DNA to host cells may be adjusted, taking into consideration such factors as the selected vector, the delivery method and the host cells selected. Introduction of the vector into a host cell may be achieved by any means known in the art or as disclosed herein, including transfection, and infection, e. g. using $CaPO_4$ transfection or electroporation.

For the construction and assembly of the desired minigene-containing recombinant adenovirus, the vector can in one example be transfected *in-vitro* in the presence of a helper virus into the packaging cell line, allowing homologous recombination to occur between the helper and the vector sequences, which permits the adenovirus-transgene sequences in the vector to be replicated and packaged into virion capsids, resulting in the recombinant viral vector particles as is well known in the art. A recombinant adenoviruses of the invention is useful e.g. in transferring a selected transgene into a selected host cell.

In a preferred embodiment of the adenovirus of the invention, the adenovirus has a seroprevalence of less than 5% in human subjects and preferably no seroprevalence in human subjects, most preferably no seroprevalence in human subjects that have not previously been in

contact with a chimpanzee adenovirus. In this context it is preferred that the human subjects belong to an ethnic group selected from Europeans, indigenous people of Africa, Asians, indigenous people of America and indigenous people of Oceania. Methods for the identification of the ethnic origin of a human subject are comprised in the art (see e.g. WO2003/102236).

In a further preferred embodiment of the recombinant adenovirus according to the invention, the adenovirus DNA is capable of entering a mammalian target cell, i.e. it is infectious. An infectious recombinant adenoviruses of the invention can be used as a vaccine and for gene therapy as also described below. Thus, in another embodiment it is preferred that the recombinant adenovirus comprises a molecule for delivery into a target cell. Preferably, the target cell is a mammalian cell, e.g. a chimpanzee cell, a rodent cell or a human cell. For example, the molecule for delivery into a target cell can be an expression cassette as defined herein. Methods to introduce an expression cassette into the genome of an adenovirus are well known in the art (see for example the literature citations provided above). In one example a recombinant adenovirus of the present invention that comprises an expression cassette, encoding e.g. a minigene or an antigene, can be generated by replacing a genomic region of the adenovirus selected from E1A, E1B, E2A, E2B, E3 and E4 with said expression cassette. The genomic regions E1A, E1B, E2A, E2B, E3 and E4 of the adenoviruses of the invention can easily be identified by an alignment with known and annotated adenoviral genomes such as from human Ad5 (see: Birgitt Täuber and Thomas Dobner, Oncogene (2001) 20, p. 7847 – 7854; and also: Andrew J. Davison, et al., "Genetic content and evolution of adenoviruses", Journal of General Virology (2003), 84, p. 2895–2908). Non-limiting examples of how to generate modified adenoviruses comprising a molecule for delivery into a target cell are also provided in examples 1 and 2 and figure 4 below.

The molecule for delivery into a target cell is preferably a polynucleotide but may also be a polypeptide or a small chemical compound, preferably having a therapeutic or diagnostic activity. In one particularly preferred embodiment, the molecule for delivery into a target cell is a polynucleotide that comprises an adenovirus 5' inverted terminal repeat sequence (ITR), a gene, e.g. SEQ ID NO: 1 and a 3' ITR. It will be evident to the skilled person that the molecular size of the molecule has to be chosen such that the capsid can form around and package the molecule, when the recombinant adenovirus is produced, e.g. in a packaging cell line. Thus, preferably the gene is a minigene which can have e.g. up to 7000 and maximally up to 8000 base pairs.

In a preferred embodiment, the molecule for delivery into a target cell comprised in the recombinant adenovirus according to the invention is a polynucleotide encoding an antigenic protein or a fragment thereof. An antigenic protein or fragment thereof is capable of eliciting an immune response in a mammal and may be in a particularly preferred embodiment the gag

protein of HIV as shown in the examples and being encoded by a polynucleotide according to SEQ ID NO: 1.

In a particularly preferred embodiment, the recombinant adenovirus of the invention is an adenovirus that has been deposited at ECACC (European Collection of Cell Culture, Porton Down, Salisbury, SP4 OJG, UK) and has a deposit number selected from the group consisting of 08110601 (ChAd83), 08110602 (ChAd73), 08110603 (ChAd55), 08110604 (ChAd147) and 08110605 (ChAd146). The deposits of the aforementioned adenoviral strains (Latin name: *Mastadenovirus, Adenoviridae*) have been made on November 6, 2008 by Okairos AG, Elisabethenstr. 3, 4051 Basel, Switzerland.

These deposits will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. These deposits were made merely as a convenience for those of skill in the art and are not an admission that a deposit is required under 35 U. S. C. 112. All restrictions on the availability to the public of the deposited material will be irrevocably removed, except for the requirements specified in 37 C. F. R. 1. 808 (b), upon the granting of a patent.

Another preferred embodiment of the recombinant adenovirus of the invention is an adenovirus derived from an adenovirus selected from the group consisting of 08110601 (ChAd83), 08110602 (ChAd73), 08110603 (ChAd55), 08110604 (ChAd147) and 08110605 (ChAd146). Preferably the adenovirus derived of one of the aforementioned deposited adenoviruses has been altered by introducing a functional deletion, deletion or modification in its genome, e.g. to obtain a replication incompetent adenovirus and/or an adenovirus that is capable of expressing a transgene in a host cell. For example, one or more genes selected from the group consisting of E1A, E1B, E2A, E2B, E3 and E4 gene can be deleted, rendered non-functional, and/or can be replaced by an expression cassette as outlined above. Additionally, one or more genes of another adenovirus may be introduced, preferably for a deleted gene. A skilled person is well aware of how to introduce these genomic alterations in the deposited strains. In this respect, methods of generating modified adenoviruses comprising a molecule for delivery into a target cell, which is a preferred modification of the deposited strains, have been described above.

In a further aspect a composition is provided that comprises an immunological adjuvant and at least one of the following (i) through (iv):

(i) an isolated protein according to the invention;

(ii) an isolated polynucleotide according to the invention;

(iii) a vector according to the invention;

(iv) a recombinant adenovirus according to the invention;

and, optionally, a pharmaceutically acceptable excipient.

A composition according to the invention that comprises an adjuvant can be used as a vaccine, e.g. for human subjects. The immunological adjuvant also referred to herein in short as "adjuvant", accelerates, prolongs and/or enhances the quality and/or strength of an immune response to an antigen/immunogen, in comparison to the administration of the antigen alone, thus, reducing the quantity of antigen/immunogen necessary in any given vaccine, and/or the frequency of injection necessary in order to generate an adequate immune response to the antigen/immunogen of interest.

Examples of adjuvants that may be used in the context of the composition according to the present invention are gel-like precipitates of aluminum hydroxide (alum); $AlPO_4$; alhydrogel; bacterial products from the outer membrane of Gram-negative bacteria, in particular monophosphoryl lipid A (MPLA), lipopolysaccharides (LPS), muramyl dipeptides and derivatives thereof; Freund's incomplete adjuvant; liposomes, in particular neutral liposomes, liposomes containing the composition and optionally cytokines; non-ionic block copolymers; ISCOMATRIX adjuvant (Drane et al., 2007); unmethylated DNA comprising CpG dinucleotides (CpG motif), in particular CpG ODN with a phosphorothioate (PTO) backbone (CpG PTO ODN) or phosphodiester (PO) backbone (CpG PO ODN); synthetic lipopeptide derivatives, in particular Pam_3Cys; lipoarabinomannan; peptidoglycan; zymosan; heat shock proteins (HSP), in particular HSP 70; dsRNA and synthetic derivatives thereof, in particular Poly I:poly C; polycationic peptides, in particular poly-L-arginine; taxol; fibronectin; flagellin; imidazoquinoline; cytokines with adjuvant activity, in particular GM-CSF, interleukin- (IL-)2, IL-6, IL-7, IL-18, type I and II interferons, in particular interferon-gamma, TNF-alpha; 25-dihydroxyvitamin D3 (calcitriol); and synthetic oligopeptides, in particular MHCII-presented peptides. Non-ionic block polymers containing polyoxyethylene (POE) and polyoxypropylene (POP), such as POE-POP-POE block copolymers may be used as an adjuvant (Newman et al., 1998). This type of adjuvant is particularly useful for compositions comprising nucleic acids as active ingredient.

Optionally, various pharmaceutically acceptable excipients may be used. Preferred pharmaceutically acceptable excipients are mentioned below when discussing the uses according to the invention.

Activation of specific receptors can stimulate an immune response. Such receptors are known to the skilled artisan and comprise, for example, cytokine receptors, in particular type I cytokine receptors, type II cytokine receptors, TNF receptors; and vitamin D receptor acting as transcription factor; and the Toll-like receptors 1 (TLR1), TLR-2, TLR 3, TLR4, TLR5, TLR-6, TLR7, and TLR9. Agonists to such receptors have adjuvant activity, i.e., are immunostimulatory. In a preferred embodiment, the adjuvant of the composition of the present invention may be one

or more Toll-like receptor agonists. In a more preferred embodiment, the adjuvant is a Toll-like receptor 4 agonist. In a particular preferred embodiment, the adjuvant is a Toll-like receptor 9 agonist, preferably being encoded by the nucleotide tccatgacgttcctgacgtt (SEQ ID NO: 2).

In a further aspect the invention provides a cell, preferably a non-simian cell, comprising at least one of the following:

(i) an isolated protein according to the invention;

(ii) an isolated polynucleotide according to the invention;

(iii) a vector according to the invention;

(iv) a recombinant adenovirus according to the invention;

The cell may be selected of a bacterial cell such as an *E. coli* cell, a yeast cell such as *Saccharomyces cerevisiae* or *Pichia pastoris*, a plant cell, an insect cell such as SF9 or Hi5 cells, or a mammalian cell. Preferred examples of mammalian cells are Chinese hamster ovary (CHO) cells, human embryonic kidney (HEK 293) cells, HELA cells, human hepatoma cells (e.g. Huh7.5), Hep G2 human hepatoma cells, Hep 3B human hepatoma cells and the like.

If the cell comprises an isolated polyucleotide according to (ii), this polynucleotide may be present in the cell either (i) freely dispersed as such, or (ii) integrated into the host cell genome or mitochondrial DNA.

In a further preferred embodiment, the cell is a host cell, preferably a 293 cell or a PER.C6™ cell, that expresses at least one adenoviral gene selected from the group consisting of E1a, E1b, E2a, E2b, E4, L1, L2, L3, L4 and L5.

Also provided is the use of the isolated polynucleotide according to the invention, the isolated protein according to the invention, the vector according to the invention, the recombinant adenovirus according to the invention and/or the pharmaceutical composition according to the invention for the therapy or prophylaxis of a disease.

Adenoviral vectors have demonstrated great potential as vaccine vectors. Preclinical and clinical studies have demonstrated the feasibility of vector design, robust antigen expression and protective immunity using this system. Thus, a preferred embodiment is the use according to the invention, wherein the therapy or prophylaxis is a vaccination, e.g. for human subjects. Detailed instructions of how adenoviruses are used and prepared for vaccination are provided as ample literature comprised in the art and known to the skilled person.

If the use is a vaccination, a recombinant adenovirus of the invention can be administered in an immunologically and/or prophylactically effective dose which is preferably 1×10^8 to 1×10^{11} viral particles (i.e., 1×10^8, 5×10^8, 1×10^9, 5×10^9, 1×10^{10}, 2.5×10^{10} or 5×10^{10} particles). Furthermore, for a vaccination which requires a boosting, it is preferred to apply a "heterologous prime-boost" methodology, as defined above. Furthermore, when using the

isolated polynucleotide according to the invention, the isolated protein according to the invention, the vector according to the invention, the recombinant adenovirus according to the invention and/or the pharmaceutical composition according to the invention in a vaccine, it is preferred that the vaccine comprises an adjuvant. Preferred immunological adjutants have been mentioned herein and can be used in such vaccine.

A recombinant adenovirus prepared using a polynucleotide or recombinant adenoviral protein or fragment thereof according to the invention can be used to transduce a host cell with a polynucleotide, e.g. DNA. Thus, a preferably replication deficient, albeit infectious, i.e. capable of entering a host cell, adenovirus can be prepared to express any custom protein or polypeptide in a host cell. Thus, in a preferred embodiment, the therapy recited in the use according to the invention is gene therapy. If an isolated polynucleotide, an isolated protein, a vector, a recombinant adenovirus and/or a pharmaceutical composition according to the invention is used for gene therapy and is administered to a subject to be treated, it is preferred that it is administered in a sufficiently large dose such that the treatment results in one or more cells of the patient being transfected, i.e. transduced. If a recombinant adenovirus and/or a pharmaceutical composition according to the invention is administered by any of the preferred means of administrations disclosed herein, it is preferred that an effective dose which is preferably 1×10^8 to 5×10^{11} viral particles (i.e., 1×10^8, 5×10^8, 1×10^9, 5×10^9, 1×10^{10}, 2.5×10^{10}, 5×10^{10}, 1×10^{11} or, most preferably, 5×10^{11} particles) is administered. In preferred embodiments, the preferably heterologous polynucleotide that is comprised in the recombinant adenovirus of the invention is capable of expressing a protein or polypeptide in a host cell of the subject, wherein the protein or polypeptide comprises a signal peptide which effects secretion of the protein or polypeptide from said host cell. For example, a patient in need of a certain protein can be treated using an adenovirus of the present invention which comprises a cDNA that encodes a secretable form of that protein.

In a further embodiment of the use of the present invention, the isolated polynucleotide, isolated protein, vector, adenovirus and/or pharmaceutical composition according to the invention (in the following referred to as pharmaceutical according to the invention) is formulated to further comprise one or more pharmaceutically acceptable diluents; carriers; excipients, including fillers, binders, lubricants, glidants, disintegrants, and adsorbents; and/or preservatives.

The pharmaceutical according to the invention can be administered by various well known routes, including oral, rectal, intragastrical and parenteral administration, e.g. intravenous, intramuscular, intranasal, intradermal, subcutaneous and similar administration routes. Parenteral-, intramuscular- and intravenous administration is preferred. Preferably the

pharmaceutical according to the invention is formulated as syrup, an infusion or injection solution, a tablet, a capsule, a capslet, lozenge, a liposome, a suppository, a plaster, a band-aid, a retard capsule, a powder, or a slow release formulation. Preferably the diluent is water, a buffer, a buffered salt solution or a salt solution and the carrier preferably is selected from the group consisting of cocoa butter and vitebesole.

Particular preferred pharmaceutical forms for the administration of the pharmaceutical according to the invention during the use of the present invention are forms suitable for injectable use and include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. Typically, such a solution or dispersion will include a solvent or dispersion medium, containing, for example, water-buffered aqueous solutions, e.g. biocompatible buffers, ethanol, polyol, such as glycerol, propylene glycol, polyethylene glycol, suitable mixtures thereof, surfactants or vegetable oils.

Infusion or injection solutions can be accomplished by any number of art recognized techniques including but not limited to addition of preservatives like anti-bacterial or anti-fungal agents, e.g. parabene, chlorobutanol, phenol, sorbic acid or thimersal. Further, isotonic agents, such as sugars or salts, in particular sodium chloride may be incorporated in infusion or injection solutions.

Preferred diluents of the present invention are water, physiological acceptable buffers, physiological acceptable buffer salt solutions or salt solutions. Preferred carriers are cocoa butter and vitebesole. Excipients which can be used with the various pharmaceutical forms of the pharmaceutical according to the invention can be chosen from the following non-limiting list:

a) binders such as lactose, mannitol, crystalline sorbitol, dibasic phosphates, calcium phosphates, sugars, microcrystalline cellulose, carboxymethyl cellulose, hydroxyethyl cellulose, polyvinyl pyrrolidone and the like;
b) lubricants such as magnesium stearate, talc, calcium stearate, zinc stearate, stearic acid, hydrogenated vegetable oil, leucine, glycerids and sodium stearyl fumarates,
c) disintegrants such as starches, croscaramellose, sodium methyl cellulose, agar, bentonite, alginic acid, carboxymethyl cellulose, polyvinyl pyrrolidone and the like.

Other suitable excipients can be found in the Handbook of Pharmaceutical Excipients, published by the American Pharmaceutical Association, which is herein incorporated by reference.

Certain amounts of the pharmaceutical according to the invention are preferred for the therapy or prophylaxis of a disease. It is, however, understood that depending on the severity of the disease, the type of the disease, as well as on the respective patient to be treated, e.g. the general health status of the patient, etc., different doses of the pharmaceutical according to the

invention are required to elicit a therapeutic or prophylactic effect. The determination of the appropriate dose lies within the discretion of the attending physician.

If the pharmaceutical according to the invention is to be used prophylactically, it may be formulated as a vaccine. In this case the pharmaceutical according to the invention is preferably administered in above outlined preferred and particular preferred doses. Preferably, the administration of the vaccine is repeated at least two, three, four, five, six, seven, eight nine or at least 10 times over the course of a defined period of time, until the vaccinated subject has generated sufficient antibodies against the pharmaceutical according to the invention so that the risk of developing the respective disease has lessened. The period of time in this case is usually variable depending on the antigenicity of the vaccine. Preferably the period of time is not more than four weeks, three months, six months or three years. In one embodiment, if an adenovirus according to the invention is used for vaccination purposes, at least one of the hyper variable domains of the hexon protein can be replaced by an immunogenic epitope of the respective disease agent that the vaccination is directed against. Vaccines typically contain one or more adjuvants as outlined above. A detailed summary of the use of adenoviruses for vaccination and methods pertaining thereto is provided in: Bangari DS and Mittal SK (2006) Vaccine, 24(7), p. 849-862; see also: Zhou D, et al., Expert Opin Biol Ther. 2006 Jan;6(1):63-72; and: Folgori A, et al., Nat Med. 2006 Feb;12(2):190-7.; see also: Draper SJ, et al., Nat Med. 2008 Aug;14(8):819-21. Epub 2008 Jul 27.

Various modifications and variations of the invention will be apparent to those skilled in the art without departing from the scope of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in the relevant fields are intended to be covered by the present invention.

The following figures are merely illustrative of the present invention and should not be construed to limit the scope of the invention as indicated by the appended claims in any way.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 Multiple sequence alignment between hexon proteins of various adenovirus isolates of the invention, using Clustal-W with default settings. Hexon proteins of said novel chimpanzee adenovirus isolates are shown (designated as PanAd1, PanAd2, PanAd3, ChAd55, ChAd73, ChAd83, ChAd146 and ChAd147). The hypervariable domains 1 through 7 are designated as "HVR 1-6" and "HVR 7", respectively.

Fig. 2 Multiple sequence alignment between fiber proteins of adenovirus ChAd55 and of further novel chimpanzee adenovirus isolates (designated as PanAd1, PanAd2, PanAd3, ChAd73, ChAd83, ChAd146 and ChAd147), using Clustal-W with default settings.

Fig. 3 Multiple sequence alignment between penton proteins of adenovirus ChAd55 and of further novel chimpanzee adenovirus isolates (designated as PanAd1, PanAd2, PanAd3, ChAd73, ChAd83, ChAd146 and ChAd147), using Clustal-W with default settings.

Fig. 4 Diagram of construction of a replication-defective adenovirus vector by homologous recombination with wild type viral genome and the corresponding shuttle plasmid. See also example 2 .

Fig. 5 Cell-mediated immune response in mice vaccinated with recombinant adenoviruses comprising an expression cassette for the expression of HIV gag protein (SEQ ID NO:1). The vaccination potency of recombinant human Ad5 and chimpanzee ChAd55 (Fig. 5A), of recombinant human Ad5 and bonobo PanAd1, PanAd2 and PanAd3 adenovirus (Fig. 5B) and of recombinant ChAd55, ChAd73, ChAd83, ChAd146 and ChAd147 was compared (Fig. 5C). The immune response was measured by Interferon-γ ELIspot assay by incubating the cells with a CD8 HIV gag epitope mapped in Balb/C mice. The results are reported as spot forming cells per 10^6 splenocytes.

Fig. 6 The seroprevalence of novel adenovirus vectors was evaluated on a panel of human sera of European origin. The seroprevalence of human adenovirus type 5 (Ad5) and of chimpanzee adenoviruses ChAd55, ChAd73, ChAd83, ChAd146, ChAd147, PanAd1, PanAd2, PanAd3 and CV-68 were evaluated in parallel on the same panel. The data are expressed as % of subjects showing an immunoprevalence. Neutralizing antibodies were only detected against Ad5 and CV-68 adenoviruses but not for any of the novel adenoviruses of the present invention.

Fig. 7 PanAd HSV immunization of BALB/c mice is shown in Fig. 7A and PanAd cancer Ag immunization of BALB/c mice is shown in Fig. 7B.

Fig. 8 PanAd HIV gag immunization of *Macaca fascicularis* is shown in a priming/boosting vaccination experiment.

EXAMPLES

Example 1: Adenovirus isolation and characterization

ChAd55, ChAd73, ChAd83, ChAd146, ChAd147 are a group of chimpanzee adenoviruses obtained from healthy animals housed in different European and US facilities. ChAd55, ChAd73, ChAd83, ChAd146, ChAd147 have the property of no detectable reactivity with human

sera. PanAd1, PanAd2 and PanAd3 are new adenovirus isolated from healthy bonobos (*Pan Paniscus*) housed in different European and US facilities. PanAd1, PanAd2 and PanAd3 have the property of no detectable reactivity with human sera.

The common chimpanzee and bonobo adenovirus stocks were cloned by infecting 293 cells seeded in 96-well plates, after the first passage of amplification. The virus cloning was performed by limiting dilution of the cell lysate obtained at the first passage of the virus amplification. 5 isolated clones were picked up and serially propagated. After 3-4 serial passages of amplification, a large-scale preparation of adenovirus was performed on cells planted on 5 two-layer cell-factories (NUNC) (200 millions of cells/cell factory). Purified viral particles were obtained from cell lysate by two ultra-centrifugation steps on cesium chloride density gradients.

Genomic DNA was isolated from 3 X 10^{12} pp of purified virus preparation by digestion with Proteinase K (0.5 mg/ml) in 1% SDS-TEN (2 hrs at 55°C). After a Phenol-Chloroform extraction and Ethanol precipitation, the genomic DNA was resuspended in water and submitted for genomic sequencing.

An initial classification of the new isolates was obtained by sequence analysis of the hypervariable region 7 (HVR7) of the hexon gene. To this end two primers were designed on the highly conserved regions flanking HVR7: TGTCCTACCARCTCTTGCTTGA (SEQ ID NO. 3) and GTGGAARGGCACGTAGCG (SEQ ID NO. 4). The HVR7 was amplified by PCR using purified viral DNA or crude 293 lysate as template and then sequenced. More detailed information about the isolate was obtained by sequencing the hypervariable regions 1 to 6. The DNA region containing HVR1-6 was amplified by PCR using oligonucleotides HVR1-6fd, CAYGATGTGACCACCGACCG (SEQ ID NO. 5) and HVR1-6rev, GTGTTYCTGTCYTGCAAGTC (SEQ ID NO. 6). Based on HVRs sequence analysis the new isolated viruses were classified into subgroup E (ChAd55, ChAd73, ChAd83, ChAd146, ChAd147) and subgroup C (PanAd1, PanAd2 and PanAd3) of human Ad virus classification (Horowitz, MS (1990), Adenoviridae and their replication. In Virology B.N. Fields and D.M. Knipe, eds (raven Press, New York) pp.1679-1740).

A phylogenetic tree was obtained by alignment of human and chimp adenovirus hexon amino acid sequences. The results are consistent with the initial classification based on nucleotide sequence alignment limited to hexon HVR1-6 and 7 by using Align X program (Informax, Inc) demonstrating a close phylogenetic relationship of ChAd55, ChAd73, ChAd83, ChAd146, ChAd147 isolates with human Ad4 (subgroup E) while bonobo adenovirus isolate PanAd1, PanAd2 and PanAd3 are related to human Ad1, 2, 5, 6 (subgroup C).

Example 2: Vector construction

The PanAd1, PanAd2 and PanAd3 and ChAd55, ChAd73, ChAd83, ChAd146, ChAd147 virus genomes were cloned in a plasmid vector following the strategy detailed below. All manipulations of the vector genome were performed in *E.coli* following standard techniques. Vector systems were developed by deleting E1 and E3 regions from ChAd and PanAd backbones. The E1 region was substituted with expression cassettes based on human CMV IE promoter and BGHpA signal containing HCV non structural region (HCV NS) and HIV gag (SEQ ID NO: 1) genes for the evaluation of the immunological potency in animal models. In addition, ChAd and PanAd vectors expressing the secreted alkaline phosphatase gene (SEAP) were constructed for the neutralization assay. The vectors were propagated in 293 cells and purified by CsCl gradients following standard protocols.

The construction of PanAd1, PanAd2 and PanAd3 ΔE1 vectors proceeded through the steps provided below.

I. Construction of PanAd Shuttle Vector

PanAd1 genome was used to construct a shuttle vector for cloning by homologous recombination the entire genome of PanAd1, PanAd2 and PanAd3. Briefly, the shuttle vector used to clone bonobo adenovirus 1 referred to herein as pBAd1RLD_EGFP was constructed as follows:

PanAd1 left end (nt 1-450) was amplified by PCR with oligonucleotides 5'- ATCTGGAATTCGTTTAAACCATCATCAATAATATACCTTATTTTG-3' (SEQ ID NO: 7) and 5'- TCAGGAACTAGTTCCGTATACCTATAATAATAAAACGGAGACTTTG-3' (SEQ ID NO: 8) digested with SpeI and EcoRI then ligated into a plasmid vector already containing HCMV-EGFP-bgh polyA cassette by generating pBAd1-L. PanAd1 right end (nt 37362-37772) was then amplified by PCR with oligonucleotides 5'-TCCAGCGGCGCGCCAGACCCGAGTCTTACCAGGA-3' (SEQ ID NO: 9) and 5'-ATTCAGGATCCGAATTCGTTTAAACCATCATCAATAATATACCTTATTTTG-3' (SEQ ID NO: 10), and cloned in pBAd1-L thus generating plasmid pBAd1-RL.

A PanAd1 DNA fragment (nt 3498-4039) containing pIX coding region was subsequently amplified by PCR with the oligonucleotides 5'-TATTCTGCGATCGCTGAGGTGGGTGAGTGGGCG -3' (SEQ ID NO: 11) and 5'-TTACTGGCGCGCCTGCCTCGAGTAAACGGCATTTGCAGGAGAAG-3' (SEQ ID NO: 12) then cloned into pBAd1-RL obtaining the plasmid pBAd1RLD EGFP shuttle. Shuttle plasmids containing the expression cassettes for secreted alkaline phosphatase (SEAP), HIV gag, HCV

non structural region (NS) genes were also constructed by substituting the EGFP gene in pBAd1RLD EGFP shuttle.

The HIV gag HCV NS region , SEAP and EGFP expression cassette based on human cytomegalovirus (HCMV) promoter and bovine growth hormone polyadenylation signal (Bgh polyA) were constructed as described in Emini et al., International Publication Number WO 03/031588. The viral DNA cassette was designed to contain restriction enzyme sites (PmeI) that are present only at the end of both ITRs to allow the release of viral DNA from plasmid DNA.

II. Construction of ΔE1 PanAd1, PanAd2 and PanAd3 Vector

PanAd1, PanAd2 and PanAd3 vectors were constructed by homologous recombination in E. coli strain BJ5183. BJ5183 cells were co-transformed with PanAd1, 2 and 3 purified viral DNAs and pBAd1RLD-EGFP or pBAd1RLD-Gag. Homologous recombination between pIX genes, right ITR DNA sequences present at the ends of linearized pBAd1RLD-EGFP or pBAd1RLD-Gag and viral genomic DNAs allowed its insertion in the plasmid vector, by deleting at the same time the E1 region that was substituted by the expression cassette. This strategy allowed for the construction of the preadeno plasmids pPanAd1, pPanAd2 and pPanAd3 expressing EGFP or HIV gag transgenes. SEAP or HCV-NS expression cassettes were then cloned into pPanAd 1, 2 and 3 vectors by replacing either EGFP or Gag expression cassettes.

III. E3 Region Deletion

A deletion of the E3 region was introduced in PanAd1, PanAd2 and PanAd3 vector backbones by using a strategy involving several steps of cloning and homologous recombination in *E.coli*. PanAd1 E3 deletion spans from nucleotide 28636 to nucleotide 32596 of genomic PanAd1 sequence (SEQ ID NO.: 13); PanAd2 E3 deletion spans from nucleotide 28653 to nucleotide 32599 of genomic PanAd2 sequence (SEQ ID NO.: 62); PanAd3 E3 deletion spans from nucleotide 28684 to nucleotide 32640 of genomic PanAd3 sequence (SEQ ID NO.: 63).

IV. E4 region Deletion

The native E4 region of PanAd1, PanAd2 and PanAd3 was deleted and replaced with Ad5 E4 ORF6 coding sequence (SEQ ID NO.: 64). The coordinates of the E4 deletion introduced in the PanAd 1, 2 and 3 backbones are the following:

PanAd1 E4 deletion spans from nucleotide 34690 to 37369 (SEQ ID NO.: 13);

PanAd2 E4 deletion spans from nucleotide 34696 to 37400. (SEQ ID NO.: 62);

PanAd3 E4 deletion spans from nucleotide 34690-37369 (SEQ ID NO.: 63).

The deleted region contains all PanAd E4 orfs while the E4 native promoter and polyadenylation signal were not deleted

The HIV gag and HCV NS region expression cassette based on human cytomegalovirus (HCMV) promoter and bovine growth hormone polyadenylation signal (Bgh polyA) was constructed as described in Emini et al., International Publication Number WO 03/031588 and inserted into PanAd1, 2 and 3 ΔE1 EGFP vector by homologous recombination in E. coli strain BJ5183 exploiting the homologies between HCMV and Bgh polyA DNA sequences.

V. ChAd55 DE1 Expression Vector Construction and Rescue

Construction of Shuttle Vector for ChAd55 cloning

ChAd55 shuttle was constructed by following the same strategy described above for PanAd vectors then used for the cloning of the ChAd55 viral genomes. To this end, the shuttle vector pARS ChAd55 containing the right end as well as the left end of viral genome (left end from the ITR to the pIX gene with the E1 region deleted and substituted with the expression cassette) was linearized with AscI restriction enzyme and co-transformed into *E.coli* strain BJ5183 with ChAd55 purified viral DNA. Homologous recombination between DNA sequences from pIX genes and right ITR present at the ends of linearized pARS ChAd55 and ChAd55, ChAd73, ChAd83, ChAd146 and ChAd147 purified viral genomic DNAs allowed their insertion into the plasmid vector by deleting at the same time the E1 region. A diagram of the chimp adenovirus 55 (ChAd55) genome cloning strategy is provided in figure 4.

Expression cassettes based on human cytomegalovirus (HCMV) promoter and bovine growth hormone poly-adenylation signal (Bgh polyA) were constructed to express secreted alkaline phosphatase (SEAP), EGFP, HIV gag , HCV NS genes. All expression cassettes were inserted into the single SnaBI site of pARS ChAd55 vector to be transferred by homologous recombination into the ΔE1 adenovirus pre-plasmids.

Example 3: Immunization experiments.

The efficiency of ChAd55, ChAd73, ChAd83, ChAd146, ChAd147, PanAd1, PanAd2 and PanAd3 vectors as potential recombinant vaccine was evaluated in mice with vectors expressing HIV gag transgene. The vector potency of ChAd55 gag was compared with human Ad5 gag in immunization experiments performed in parallel. Groups of 10 animals were injected in the quadriceps with a dose of the vector of 10^8 vp/mouse for Ad5gag or ChAd55gag (Fig. 5A). In a separate experiment a group of 5 animals were injected with a dose of the vector of 10^8 vp/mouse for Ad5gag or PanAd1gag, PanAd2gag and PanAd3gag (Fig. 5B). The potency of

ChAd73 gag, ChAd83 gag, ChAd146 gag and Chad147gag was also determined by immunizing groups of 5 mice with a dose of vector of 10^8 vp/mouse in parallel with ChAd55 gag (Fig. 5C). The immune response elicited against HIV gag was measured by Interferon-γ Elispot assay on splenocytes. The results of immunization experiments with ChAd55, ChAd73, ChAd83, ChAd146, ChAd147 and PanAd1, PanAd2 and PanAd3 in comparison with human Ad5 gag vector show that the novel adenoviruses of the invention are at least as effective in eliciting a specific immune response as the prior art recombinant adenovirus Ad5.

Example 4: Neutralization Studies

Neutralization assays were carried out in order to evaluate the prevalence in human sera of neutralizing antibodies against the common chimpanzee adenovirus 55, 73, 83, 146, 147 and the Bonobo adenovirus type 1, 2 and 3. The assay evaluated the effects of serum preincubation on the ability of ChAd55, ChAd73, ChAd83, ChAd146, ChAd147, PanAd1, PanAd2 and PanAd3 carrying the gene for secreted alkaline phosphatase (SEAP) to transduce human 293 cells. The neutralization titer is defined as the dilution of serum giving a 50% reduction of the SEAP activity observed in the positive control with the virus in absence of serum. Each serum sample was tested at various dilutions (five 4-fold increments starting from 1/18 dilution through 1:4608). Samples were pre-incubated for one hour at 37°C and then added to 293 cells seeded into 96-well plates ($3x10^4$ cells/well). A panel of human sera was tested for neutralization activity. In parallel the same panel was tested on Ad5 and on chimp and bonobo Ad SEAP vectors. The results are provided in Figure 6. The results indicate that the seroprevalence on chimpanzee adenoviruses is lower than human adenovirus Ad5. However, in general the presence of neutralizing antibodies against already described ChAds (CV-68) can be detected in a subset of subjects. On the contrary, all human sera tested so far failed to neutralize ChAd55 and PanAd1, PanAd2 and PanAd3 even at very low titer. The same was observed for ChAd73, ChAd83, ChAd146 and ChAd147. Therefore, the novel adenovirus isolates ChAd55, ChAd73, ChAd83, ChAd146, ChAd147 and PanAd1, PanAd2 and PanAd3 represent the ideal solution to the problem of the pre-existing anti-human Ad immunity that limits the administration of viral vectors based on common human Ad serotypes such as Ad5.

Example 5: Immunization efficiency of PanAd1 and 3 vectors in comparison with Ad5 vectors

The efficiency of PanAd1 and PanAd3 vectors as potential recombinant vaccines was evaluated in BALB/c mice with vectors expressing herpes simplex virus (HSV) antigen and with vectors

expressing a cancer antigen. The vector potency of PanAd1 and 3 expressing HSV Ag and the cancer Ag was compared with the corresponding vectors based on human Ad5.

To evaluate the antiviral potency, 9 groups of BALB/c mice were injected in the quadriceps with increasing doses of the vectors starting from 10^7 vp/mouse up to 10^9 vp/mouse in parallel with PanAd1-HSV, PanAd3-HSV and Ad5-HSV (see Fig. 7A). The immune response elicited against the HSV antigen was measured by Interferon-γ Elispot assay on mouse splenocytes incubated with a peptide pool covering the entire amino acid sequence of the antigen. The results of immunization experiments with PanAd1, PanAd2 and PanAd3 in comparison with human Ad5 vector reported in Figure 7 showed that the novel adenoviruses of the invention are more effective in eliciting a specific immune response than the prior art recombinant adenovirus Ad5 at each concentration tested. This is clearly demonstrated by the higher frequency of antigen-specific T-cell observed in mice immunized with PanAd1 and PanAd3 vectors.

The efficiency in eliciting anti-tumoral T-cell response by PanAd vectors was evaluated by immunizing groups of BALB/c mice by injecting in the quadriceps increasing doses of the vectors starting from 10^7 vp/mouse up to 10^9 vp/mouse. Two groups of BALB/C mice were injected with Ad5 vector expressing the tumor antigen at 10^7 vp/mouse and 10^9 vp/mouse. In parallel 3 groups of BALB/c mice were immunized with 10^7, 10^8 ,10^9 vp of PanAd1 or PanAd3 vectors carrying the same tumor antigen. The T cell response was measured by Interferon-γ Elispot assay on splenocytes using a single peptide representing a mapped CD8 epitope. The results shown in Figure 7B demonstrated a higher frequency of responding animals at the lowest dose of the vaccine as well as a higher frequency of antigen-specific T-cell in the groups of animals immunized with the PanAd vectors in comparison with those immunized with Ad5 vector.

Example 6: Immunization of Macaca fascicularis with PanAd vectors

Two groups of 3 macaques were immunized by intramuscular injection of CsCl-purified PanAd1 and PanAd3 in a heterologous prime/boost regimen. Each animal in the group 1 received a dose of 10^8 vp while the animals in the group 2 received a dose of 10^{10} vp of PanAd3 Gag vector in the deltoid muscle at week 0. All animals in both groups were than boosted with a single dose of PanAd1 Gag of 10^{10} vp at week 13.

CMI was measured at different time points by IFN-γ ELISPOT assay. This assays measure HIV antigen-specific CD8+ and CD4+ T lymphocyte responses. Peptides based on the amino acid sequence of HIV Gag protein were prepared for use in these assays to measure immune responses in adenovirus vector vaccinated monkeys. The individual peptides are overlapping 20-mers, offset by 10 amino acids.

The IFNγ-ELISPOT assay provides a quantitative determination of antigen-specific T lymphocyte responses. PBMC are serially diluted and placed in microplate wells coated with anti-rhesus IFN-γ antibody (MD-1 U-Cytech). They are cultured with a HIV Gag peptide pool for 20 hours, resulting in the restimulation of the precursor cells and secretion of IFN-γ. The cells are washed away, leaving the secreted IFN bound to the antibody-coated wells in concentrated areas where the cells were sitting. The captured IFN is detected with biotinylated anti-rhesus IFN antibody (detector Ab U-Cytech) followed by alkaline phosphatase-conjugated streptavidin (Pharmingen 13043E). The addition of insoluble alkaline phosphatase substrate results in dark spots in the wells at the sites where the cells were located, leaving one spot for each T cell that secreted IFN-γ.

The number of spots per well is directly related to the precursor frequency of antigen-specific T cells. Gamma interferon was selected as the cytokine visualized in this assay (using specific anti-gamma interferon monoclonal antibodies) because it is the most common, and one of the most abundant cytokines synthesized and secreted by activated T lymphocytes. For this assay, the number of spot forming cells (SFC) per million PBMCs is determined for samples in the presence and absence (media control) of peptide antigens. Data from macaques on PBMC obtained at different time points post dose 1 and post dose 2 are shown in Figure 8. All animals primed with PanAd3 at both doses showed a T cell response against HIV Gag, efficiently boosted by the second injection of PanAd1 demonstrating that, as already suggested by the hexon, penton and fiber sequence alignment, PanAd1 and PanAd3 are distinct serotypes that can be combined in a heterologous prime-boost immunization regimen. Thus, in another aspect the invention provides the use of two recombinant adenoviruses of the invention for a heterologous prime-boost immunization wherein the two recombinant adenoviruses of the invention are of distinct adenoviral serotypes, most preferably of PanAd1 and PanAd3 as described herein.

Applicant's or agent's file reference 578-13 PCT 2	International application No. PCT/EP2010/000 616

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Date of deposit	Accession Number
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C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

ChAd55 - Mastadenovirus, Adenoviridae

Additional Request:
The applicant hereby declares that the availability of the bilogical material as stated above shall be effected only by the issue of a sample to an expert.

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ChAd73 - Mastadenovirus, Adenoviridae

Additional Request:
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ChAd83 - Mastadenovirus, Adenoviridae

Additional Request:
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ChAd146 - Mastadenovirus, Adenoviridae

Additional Request:
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ChAd147 - Mastadenovirus, Adenoviridae

Additional Request:
The applicant hereby declares that the availability of the bilogical material as stated above shall be effected only by the issue of a sample to an expert.

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CLAIMS

1. An isolated polynucleotide that encodes an adenoviral fiber protein or a functional derivative thereof and that is selected from the group consisting of:
   (a) a polynucleotide encoding a polypeptide having the amino acid sequence according to any of SEQ ID NOs: 14-19, 50 and 53;
   (b) a polynucleotide encoding the functional derivative of a polypeptide according to any of SEQ ID NOs: 14-19, 50 and 53, wherein said functional derivative comprises the deletion, insertion and/or substitution of one or more amino acid residues; and
   (c) a polynucleotide encoding a functional derivative having an amino acid sequence which is at least 85% identical over its entire length to the amino acid sequence of any of SEQ ID NOs: 14-19, 50 and 53.

2. An isolated polynucleotide that encodes an adenoviral hexon protein or a functional derivative thereof and that is selected from the group consisting of:
   (a) a polynucleotide encoding a polypeptide having the amino acid sequence according to any of SEQ ID NOs: 20-25, 51 and 54;
   (b) a polynucleotide encoding the functional derivative of a polypeptide according to any of SEQ ID NOs: 20-25, 51 and 54, wherein said functional derivative comprises the deletion, insertion and/or substitution of one or more amino acid residues; and
   (c) a polynucleotide encoding a functional derivative having an amino acid sequence which is at least 95% identical over its entire length to the amino acid sequence of any of SEQ ID NOs: 20-25, 51 and 54.

3. An isolated polynucleotide that encodes an adenoviral penton protein or a functional derivative thereof and that is selected from the group consisting of:
   (a) a polynucleotide encoding a polypeptide having the amino acid sequence according to any of SEQ ID NOs: 26-31, 52 and 55;
   (b) a polynucleotide encoding the functional derivative of a polypeptide according to any of SEQ ID NOs: 26-31, 52 and 55, wherein said functional derivative comprises the deletion, insertion and/or substitution of one or more amino acid residues; and

(c) a polynucleotide encoding a functional derivative having an amino acid sequence which is at least 85% identical over its entire length to the amino acid sequence of any of SEQ ID NOs: 26-31, 52 and 55.

4. A polynucleotide comprising at least one isolated polynucleotide according to any of claims 1, 2 and 3.

5. The isolated polynucleotide according to any of claims 1-4, wherein the polynucleotide comprises at least one of the following:

(a) an adenoviral 5'-end, preferably an adenoviral 5' inverted terminal repeat;

(b) an adenoviral Ela region, or a fragment thereof selected from among the 13S, 12S and 9S regions;

(c) an adenoviral Elb region, or a fragment thereof selected from among the group consisting of the small T, large T and IX regions;

(d) an adenoviral E2b region; or a fragment thereof selected from among the group consisting of the small pTP, Polymerase and IVa2 regions;

(e) an adenoviral L1 region, or a fragment thereof, said fragment encoding an adenoviral protein selected from the group consisting of the 28.1 kD protein, polymerase, agnoprotein, 52/55 kDa protein, and IIIa protein;

(f) an adenoviral L2 region, or a fragment thereof, said fragment encoding an adenoviral protein selected from the group consisting of the penton protein according to claim 3, VII, V, and Mu protein;

(g) an adenoviral L3 region, or a fragment thereof, said fragment encoding an adenoviral protein selected from the group consisting of the VI protein, hexon protein according to claim 2 and endoprotease;

(h) an adenoviral E2a region;

(i) an adenoviral L4 region, or a fragment thereof said fragment encoding an adenoviral protein selected from the group consisting of the 100 kD protein, the 33 kD homolog, and protein VIII;

(j) an adenoviral E3 region, or a fragment thereof selected from the group consisting of E3 ORF1, E3 ORF2, E3 ORF3, E3 ORF4, E3 ORF5, E3 ORF6, E3 ORF7, E3 ORF8, and E3 ORF9;

(k) an adenoviral L5 region, or a fragment thereof said fragment encoding the fiber protein according to claim 1;

(l) an adenoviral E4 region, or a fragment thereof selected from the group consisting of E4 ORF7, E4 ORF6, E4 ORF5, E4 ORF4, E4 ORF3, E4 ORF2, and E4 ORF1 ; and /or

(m) an adenoviral 3'-end, preferably an adenoviral 3' inverted terminal repeat.

6. The isolated polynucleotide according to claim 4, wherein the polynucleotide consists of or comprises a polynucleotide which is at least 90% identical over its entire length to a sequence that essentially consists of any one of SEQ ID NO: 13, 62, 63 or 65 or to a sequence that consists of any one of SEQ ID NO: 13, 62, 63 or 65 but lacks the genomic regions E1A, E1B, E2A, E2B, E3 and/or E4 of SEQ ID NO: 13, 62, 63 or 65.

7. An isolated adenoviral capsid polypeptide encoded by the isolated polynucleotide according to any of claims 1-3, or a functional derivative thereof.

8. A vector comprising the isolated polynucleotide according to any of claims 1-6.

9. The vector according to claim 8, wherein the vector does not comprise a gene in a genomic region selected from the group of genomic regions consisting of E1A, E1B, E2A, E2B, E3 and E4, and/or comprises at least one gene of a genomic region selected from the group of E1A, E1B, E2A, E2B, E3 and E4, wherein said at least one gene comprises a deletion and/or mutation which renders the at least one gene non-functional.

10. A recombinant adenovirus, preferably a replication-incompetent adenovirus, comprising an isolated polynucleotide according to any of claims 1-6 and/or at least one isolated adenoviral capsid polypeptide according to claim 7.

11. The recombinant adenovirus of claim 10, wherein the recombinant adenovirus comprises a molecule for delivery into a target cell.

12. The recombinant adenovirus according to claim 10 or 11, wherein the adenovirus has a seroprevalence of less than 5% in human subjects and preferably no seroprevalence in human subjects.

13. The recombinant adenovirus according to any of claims 10-12, wherein the adenovirus is capable of infecting a mammalian cell.

14. The recombinant adenovirus according to any of claims 11-13, wherein the molecule for delivery into a target cell is a polynucleotide encoding an antigenic protein or a fragment thereof.

15. The recombinant adenovirus of any of claims 10-14, wherein the adenovirus is an adenovirus that has been deposited and has a deposit number selected from the group consisting of 08110601 (ChAd83), 08110602 (ChAd73), 08110603 (ChAd55), 08110604 (ChAd147) and 08110605 (ChAd146).

16. A composition comprising an adjuvant and at least one of the following (i) through (iv):
(i) one or more isolated adenoviral capsid polypeptides according to claim 7;
(ii) an isolated polynucleotide according to any of claims 1-6;
(iii) a vector according to any of claims 8-9;
(iv) a recombinant adenovirus according to any of claims 10-15;
and, optionally, a pharmaceutically acceptable excipient

17. The composition according to claim 16, wherein the adjuvant is an agonist for a receptor selected from the group consisting of type I cytokine receptors, type II cytokine receptors, TNF receptors, vitamin D receptor acting as transcription factor, and the Toll-like receptors 1 (TLR1), TLR-2, TLR 3, TLR4, TLR5, TLR-6, TLR7 and TLR9.

18. The composition according to claim 17, wherein the adjuvant is a Toll-like receptor 4 or 9 agonist.

19. A cell comprising at least one of the following:
(i) one or more isolated adenoviral capsid polypeptides according to claim 7;
(ii) an isolated polynucleotide according to any of claims 1-6;
(iii) a vector according to any of claims 8-9;

(iv) a recombinant adenovirus according to any of claims 10-15;

20. The cell according to claim 18, wherein the cell is a host cell that expresses at least one adenoviral gene selected from the group consisting of E1a, E1b, E2a, E2b, E4, L1, L2, L3, L4 and L5.

21. Use of an isolated adenoviral capsid polypeptides according to claim 7; an isolated polynucleotide according to any of claims 1-6; a vector according to any of claims 8-9; a recombinant adenovirus according to any of claims 10-15; and/or the composition according to claim 18 for the therapy or prophylaxis of a disease.

22. Use according to claim 21, wherein the therapy or prophylaxis is a vaccination.

23. Use according to claim 21, wherein the therapy is gene therapy.

Figure 1

Adenovirus Hexon Protein

CLUSTAL W (1.83) multiple sequence alignment

```
PanAd1    MATPSMMPQWSYMHISGQDASEYLSPGLVQFARATDSYFSLSNKFRNPTVAPTHDVTTDR
PanAd2    MATPSMMPQWSYMHISGQDASEYLSPGLVQFARATDSYFSLSNKFRNPTVAPTHDVTTDR
PanAd3    MATPSMMPQWSYMHISGQDASEYLSPGLVQFARATDSYFSLSNKFRNPTVAPTHDVTTDR
ChAd55    MATPSMLPQWAYMHIAGQDASEYLSPGLVQFARATDTYFSLGNKFRNPTVAPTHDVTTDR
ChAd73    MATPSMLPQWAYMHIAGQDASEYLSPGLVQFARATDTYFSLGNKFRNPTVAPTHDVTTDR
ChAd83    MATPSMLPQWAYMHIAGQDASEYLSPGLVQFARATDTYFSLGNKFRNPTVAPTHDVTTDR
ChAd146   MATPSMLPQWAYMHIAGQDASEYLSPGLVQFARATDTYFSLGNKFRNPTVAPTHDVTTDR
ChAd147   MATPSMLPQWAYMHIAGQDASEYLSPGLVQFARATDTYFSLGNKFRNPTVAPTHDVTTDR
          ******:***:****:********************:****.******************

PanAd1    SQRLTLRFIPVDREDTAYSYKARFTLAVGDNRVLDMASTYFDIRGVLDRGPTFKPYSGTA
PanAd2    SQRLTLRFIPVDREDTAYSYKARFTLAVGDNRVLDMASTYFDIRGVLDRGPTFKPYSGTA
PanAd3    SQRLTLRFIPVDREDTAYSYKARFTLAVGDNRVLDMASTYFDIRGVLDRGPTFKPYSGTA
ChAd55    SQRLTLRFVPVDREDNTYSYKVRYTLAVGDNRVLDMASTYFDIRGVLDRGPSFKPYSGTA
ChAd73    SQRLTLRFVPVDGEDNTYSYKVRYTLAVGDNRVLDMASTYFDIRGVLDRGPSFKPYSGTA
ChAd83    SQRLTLRFVPVDREDNTYSYKVRYTLAVGDNRVLDMASTYFDIRGVLDRGPSFKPYSGTA
ChAd146   SQRLTLRFVPVDREDNTYSYKVRYTLAVGDNRVLDMASTYFDIRGVLDRGPSFKPYSGTA
ChAd147   SQRLTLRFVPVDREDNTYSYKVRYTLAVGDNRVLDMASTYFDIRGVLDRGPSFKPYSGTA
          ********:*** **.:****.*:****************************:********

                    <----------------------------- HVR 1-6 ------
PanAd1    YNSLAPKGAPNSCEWEQVEP--AEEAAENE-DEEEEEDVVDPQEQEPTTKTHVYAQAPLS
PanAd2    YNSLAPKGAPNPCEWDEAVT--AVDINLDELGEDEDDAEGEAEQQ----KSHVFGQAPYS
PanAd3    YNSLAPKGAPNSCEWEQEETQTAEEAQDEEEDEAEAEEEMPQEEQAPVKKTHVYAQAPLS
ChAd55    YNSLAPKGAPNTSQWITKDN-------------------------GTDKTYSFGNAPVR
ChAd73    YNALAPKGAPNTSQWITKDN-------------------------GTDKTYSFGNAPVR
ChAd83    YNSLAPKGAPNTSQWITKDN-------------------------GTDKTYSFGNAPVR
ChAd146   YNSLAPKGAPNTSQWITKDN-------------------------GTDKTYSFGNAPVR
ChAd147   YNSLAPKGAPNTSQWVTKDN-------------------------GTDKTYSFGNAPVR
          **:********..:*                                  *:: :.:**

          -------------------- HVR 1-6 -------------------------------
PanAd1    GEKITKDGLQIGTEATAAGGTKDLFADPTFQPEPQVGESQWNEAD--ATAAGGRVLKKTT
PanAd2    GQNITKEGIQIGVDTTSQA-QTPLYADKTFQPEPQVGESQWNETE--INYGAGRVLKKTT
PanAd3    GEKITKDGLQIGTDATATE-QKPIYADPTFQPEPQIGESQWNEAD--ASVAGGRVLKKTT
ChAd55    GLDITEEGLQIGPDESGGE-SKKIFADKTYQPEPQLGDEEWHDTIGAEDKYGGRALKPAT
ChAd73    GLDITEEGLQIRTDESGGE-SKKIFADKTYQPEPQLGDEEWHDTIGAEDKYGGRALKPAT
ChAd83    GLDITEEGLQIGTDESGGE-SKKIFADKTYQPEPQLGDEEWHDTIGAEDKYGGRALKPAT
ChAd146   GLDITEEGLQIGTDESGGK-SKKIFADKTYQPEPQLGDEEWHDTIGAEDKYGGRALKPAT
ChAd147   GLDITEEGLQIGTDDSSTE-SKKIFADKTYQPEPQVGDEEWHDTIGAEDKYGGRALKPAT
          * .**::*:**  : :.    . ::** *:*****:*:.:*:::       .**.** :*



          -------------------- HVR 1-6 -------------------------------
PanAd1    PMKPCYGSYARPTNANGGQGVLKANAQGVLESQVEMQFFSTSTNATN-EQNNIQPKLVLY
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PanAd2    LMKPCYGSYARPTNENGGQGILLEKEGGKPESQVEMQFFSTTQAAAAGNSDNLTPKVVLY
PanAd3    PMKPCYGSYARPTNANGGQGVLVEKDGGKMESQVDMQFFSTSENARN-EANNIQPKLVLY
ChAd55    NMKPCYGSFAKPTNAKGGQAKSRTKDDGTTEPDIDMAFFDDRSQQAS-----FSPELVLY
ChAd73    NMKPCYGSFAKPTNAKGGQAKSRTKDDGTTEPDIDMAFFDDRSQQAS-----FSPELVLY
ChAd83    NMKPCYGSFAKPTNAKGGQAKSRTKDDGTTEPDIDMAFFDDRSQQAS-----FSPELVLY
ChAd146   NMKPCYGSFAKPTNAKGGQAKSRTKDDGTTEPDIDMAFFDDRSQQAS-----FSPELVLY
ChAd147   NMKPCYGSFAKPTNAKGGQAKTRTKDDGTTEPDIDMAFFDDRSQQAS-----FSPELVLY
          *******:*:*** :***.    :  *  *.:::* **.           : *::***

          --- HVR 1-6 ------------->
PanAd1    SEDVHMETPDTHISYKPTKSDDNSKVMLGQQSMPNRPNYIAFRDNFIGLMYYNSTGNMGV
PanAd2    SEDVHLETPDTHISYMPTSNEANSRELLGQQAMPNRPNYIAFRDNFIGLMYYNSTGNMGV
PanAd3    SEDVHMETPDTHISYKPAKSDDNSKVMLGQQSMPNRPNYIGFRDNFIGLMYYNSTGNMGV
ChAd55    TENVDLDTPDTHIIYKPGTDETSSSFNLGQQSMPNRPNYIGFRDNFIGLMYYNSTGNMGV
ChAd73    TENVDLDTPDTHIIYKPGTDETSSSFNLGQQSMPNRPNYIGFRDNFIGLMYYNSTGNMGV
ChAd83    TENVDLDTPDTHIIYKPGTDETSSSFNLGQQSMPNRPNYIGFRDNFIGLMYYNSTGNMGV
ChAd146   TENVDLDTPDTHIIYKPGTDETSSSFNXGQQSMPNRPNYIGFRDNFIGLMYYNSTGNMGV
ChAd147   TENVDLETPDTHIIYKPGTDETSSSFNLGQQSMPNRPNYIGFRDNFIGLMYYNSTGNMGV
          :*:*.::****** * * ..: .*    ***:********.******************

PanAd1    LAGQASQLNAVVDLQDRNTELSYQLLLDSMGDRTRYFSMWNQAVDSYDPDVRIIENHGTE
PanAd2    LAGQASQLNAVVDLQDRNTELSYQLLLDSMGDRTRYFSMWNQAVDSYDPDVRIIENHGTE
PanAd3    LAGQASQLNAVVDLQDRNTELSYQLLLDSMGDRTRYFSMWNQAVDSYDPDVRIIENHGTE
ChAd55    LAGQASQLNAVVDLQDRNTELSYQLLLDSLGDRTRYFSMWNQAVDSYDPDVRIIENHGVE
ChAd73    LAGQASQLNAVVDLQDRNTELSYQLLLDSLGDRTRYFSMWNQAVDSYDPDVRIIENHGVE
ChAd83    LAGQASQLNAVVDLQDRNTELSYQLLLDSLGDRTRYFSMWNQAVDSYDPDVRIIENHGVE
ChAd146   LAGQASQLNAVVDLQDRNTELSYQLLLDSLGDRTRYFSMWNQAVDSYDPDVRIIENHGVE
ChAd147   LAGQASQLNAVVDLQDRNTELSYQLLLDSLGDRTRYFSMWNQAVDSYDPDVRIIENHGVE
          ****************************:****************************.*

                    <---------- HVR 7 ------------------------->
PanAd1    DELPNYCFPLGGIGITDTYQAIKTNG-NGAGDQATTWQKDSQFADRNEIGVGNNFAMEIN
PanAd2    DELPNYCFPLGGIINTETLTKVKP-----KTGQDAQWEKDTEFSEKNEIRVGNNFAMEIN
PanAd3    DELPNYCFPLGGIGVTDTYQAIKTNG-NGNGGGNTTWTKDETFADRNEIGVGNNFAMEIN
ChAd55    DELPNYCFPLNGVGFTDTFQGIKVKTTNNGTANATEWESDTSVNNANEIAKGNPFAMEIN
ChAd73    DELPNYCFPLNGVGFTDTFQGIKVKTTNNGTANATEWESDTSVNNANEIAKGNPFAMEIN
ChAd83    DELPNYCFPLNGVGFTDTFQGIKVKTTNNGTANATEWESDTSVNNANEIAKGNPFAMEIN
ChAd146   DELPNYCFPLNGVGFTDTFQGIKVKTTNNGTANATEWESDTSVNNANEIAKGNPFAMEIN
ChAd147   DELPNYCFPLNGVGFTDTFQGIKVKTTNNGTANATEWESDTSVNNANEIAKGNPFAMEIN
          **********.*:  *:*   :*          : * .*  . : ***  ** ******

PanAd1    LSANLWRNFLYSNVALYLPDKLKYNPSNVEISDNPNTYDYMNKRVVAPGLVDCYINLGAR
PanAd2    LNANLWRNFLYSNVALYLPDKLKYTPANVQISSNSNSYDYMNKRVVAPGLVDCYINLGAR
PanAd3    LSANLWRNFLYSNVALYLPDKLKYNPSNVEISDNPNTYDYMNKRVVAPGLVDCYINLGAR
ChAd55    IQANLWRNFLYANVALYLPDSYKYTPANITLPTNTNTYDYMNGRVVAPSLVDAYINIGAR
ChAd73    IQANLWRNFLYANVALYLPDSYKYTPANITLPTNTNTYDYMNGRVVAPSLVDAYINIGAR
ChAd83    IQANLWRNFLYANVALYLPDSYKYTPANITLPTNTNTYDYMNGRVVAPSLVDAYINIGAR
ChAd146   IQANLWRNFLYANVALYLPDSYKYTPANITLPTNTNTYDYMNGRVVAPSLVDAYINIGAR
ChAd147   IQANLWRNFLYANVALYLPDSYKYTPANVTLPTNTNTYEYMNGRVVAPSLVDSYINIGAR
          :.*********:********. **.*:*: :. *.*:*:*** *****.***.***:***

PanAd1    WSLDYMDNVNPFNHHRNAGLRYRSMLLGNGRYVPFHIQVPQKFFAIKNLLLLPGSYTYEW
PanAd2    WSLDYMDNVNPFNHHRNAGLRYRSMLLGNGRYVPFHIQVPQKFFAIKNLLLLPGSYTYEW
PanAd3    WSLDYMDNVNPFNHHRNAGLRYRSMLLGNGRYVPFHIQVPQKFFAIKNLLLLPGSYTYEW
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ChAd55    WSLDPMDNVNPFNHHRNAGLRYRSMLLGNGRYVPFHIQVPQKFFAIKSLLLLPGSYTYEW
ChAd73    WSLDPMDNVNPFNHHRNAGLRYRSMLLGNGRYVPFHIQVPQKFFAIKSLLLLPGSYTYEW
ChAd83    WSLDPMDNVNPFNHHRNAGLRYRSMLLGNGRYVPFHIQVPQKFFAIKSLLLLPGSYTYEW
ChAd146   WSLDPMDNVNPFNHHRNAGLRYRSMLLGNGRYVPFHIQVPQKFFAIKNLLLLPGSYTYEW
ChAd147   WSLDPMDNVNPFNHHRNAGLRYRSMLLGNXRFVPFHIQVPQKFFAIKSLLLLPGSYTYEW
          **** ************************ *:***************.************

PanAd1    NFRKDVNMVLQSSLGNDLRVDGASIKFESICLYATFFPMAHNTASTLEAMLRNDTNDQSF
PanAd2    NFRKDVNMVLQSSLGNDLRVDGASIKFESICLYATFFPMAHNTASTLEAMLRNDTNDQSF
PanAd3    NFRKDVNMVLQSSLGNDLRVDGASIKFESICLYATFFPMAHNTASTLEAMLRNDTNDQSF
ChAd55    NFRKDVNMILQSSLGNDLRTDGASIAFTSINLYATFFPMAHNTASTLEAMLRNDTNDQSF
ChAd73    NFRKDVNMILQSSLGNDLRTDGASIAFTSINLYATFFPMAHNTASTLEAMLRNDTNDQSF
ChAd83    NFRKDVNMILQSSLGNDLRTDGASIAFTSINLYATFFPMAHNTASTLEAMLRNDTNDQSF
ChAd146   NFRKDVNMILQSSLGNDLRTDGASIAFTSINLYATFFPMAHNTASTLEAMLRNDTNDQSF
ChAd147   NFRKDVNMILQSSLGNDLRTDGASISFTSINLYATFFPMAHNTASTLEAMLRNDTNDQSF
          ********:**********.***** * ** ****************************

PanAd1    NDYLSAANMLYPIPANATNVPISIPSRNWAAFRGWAFTRLKTKETPSLGSGFDPYYTYSG
PanAd2    NDYLSAANMLYPIPANATNVPISIPSRNWAAFRGWAFTRLKTKETPSLGSGFDPYYTYSG
PanAd3    NDYLSAANMLYPIPANATNVPISIPSRNWAAFRGWAFTRLKTKETPSLGSGFDPYYTYSG
ChAd55    NDYLSAANMLYPIPANATNVPISIPSRNWAAFRGWSFTRLKTRETPSLGSGFDPYFVYSG
ChAd73    NDYLSAANMLYPIPANATNVPISIPSRNWAAFRGWSFTRLKTRETPSLGSGFDPYFVYSG
ChAd83    NDYLSAANMLYPIPANATNVPISIPSRNWAAFRGWSFTRLKTRETPSLGSGFDPYFVYSG
ChAd146   NDYLSAANMLYPIPANATNVPISIPSRNWAAFRGWSFTRLKTRETPSLGSGFDPYFVYSG
ChAd147   NDYLSAANMLYPIPANATNVPISIPSRNWAAFRGWSFTRLKTKETPSLGSGFDPYFVYSG
          ************************************:******:************:.***

PanAd1    SIPYLDGTFYLNHTFKKVSVTFDSSVSWPGNDRLLTPNEFEIKRSVDGEGYNVAQCNMTK
PanAd2    SIPYLDGTFYLNHTFKKVSVTFDSSVSWPGNDRLLTPNEFEIKRSVDGEGYNVAQCNMTK
PanAd3    SIPYLDGTFYLNHTFKKVSVTFDSSVSWPGNDRLLTPNEFEIKRSVDGEGYNVAQCNMTK
ChAd55    SIPYLDGTFYLNHTFKKVSITFDSSVSWPGNDRLLTPNEFEIKRTVDGEGYNVAQCNMTK
ChAd73    SIPYLDGTFYLNHTFKKVSITFDSSVSWPGNDRLLTPNEFEIKRTVDGEGYNVAQCNMTK
ChAd83    SIPYLDGTFYLNHTFKKVSITFDSSVSWPGNDRLLTPNEFEIKRTVDGEGYNVAQCNMTK
ChAd146   SIPYLDGTFYLNHTFKKVSITFDSSVSWPGNDRLLTPNEFEIKRTVDGEGYNVAQCNMTK
ChAd147   SIPYLDGTFYLNHTFKKVSITFDSSVSWPGNDRLLTPNEFEIKRTVDGEGYNVAQCNMTK
          ********************:************************:***************

PanAd1    DWFLIQMLANYNIGYQGFYIPESYKDRMYSFFRNFQPMSRQVVDETKYKDYQQVGIIHQH
PanAd2    DWFLIQMLANYNIGYQGFYIPESYKDRMYSFFRNFQPMSRQVVDETKYKDYQQVGIIHQH
PanAd3    DWFLIQMLANYNIGYQGFYIPESYKDRMYSFFRNFQPMSRQVVDETKYKDYQQVGIIHQH
ChAd55    DWFLVQMLAHYNIGYQGFYVPEGYKDRMYSFFRNFQPMSRQVVDEVNYKDYQAVTLAYQH
ChAd73    DWFLVQMLAHYNIGYQGFYVPEGYKDRMYSFFRNFQPMSRQVVDEVNYKDYQAVTLAYQH
ChAd83    DWFLVQMLAHYNIGYQGFYVPEGYKDRMYSFFRNFQPMSRQVVDEVNYKDYQAVTLAYQH
ChAd146   DWFLVQMLAHYNIGYQGFYVPEGYKDRMYSFFRNFQPMSRQVVDEVNYKDYQAVTLAYQH
ChAd147   DWFLVQMLAHYNIGYQGFYVPEGYKDRMYSFFRNFQPMSRQVVDEVNYKDYQAVTLAYQH
          ****:****:**********:**.**********************.:***** * : :**

PanAd1    NNSGFVGYLAPTMREGQAYPANFPYPLIGKTAVDSITQKKFLCDRTLWRIPFSSNFMSMG
PanAd2    NNSGFVGYLAPTMREGQAYPANFPYPLIGKTAVDSVTQKKFLCDRTLWRIPFSSNFMSMG
PanAd3    NNSGFVGYLAPTMREGQAYPANFPYPLIGKTAVDSVTQKKFLCDRTLWRIPFSSNFMSMG
ChAd55    NNSGFVGYLAPTMRQGQPYPANYPYPLIGKSAVASVTQKKFLCDRVMWRIPFSSNFMSMG
ChAd73    NNSGFVGYLAPTMRQGQPYPANYPYPLIGKSAVASVTQKKFLCDRVMWRIPFSSNFMSMG
ChAd83    NNSGFVGYLAPTMRQGQPYPANYPYPLIGKSAVASVTQKKFLCDRVMWRIPFSSNFMSMG
ChAd146   NNSGFVGYLAPTMRQGQPYPANYPYPLIGKSAVASVTQKKFLCDRVMWRIPFSSNFMSMG
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ChAd147   NNSGFVGYLAPTMRQGQPYPANYPYPLIGKSAVTSVTQKKFLCDRVMWRIPFSSNFMSMG
          **************:**.****:*******:** *:*********.:*************

PanAd1    ALTDLGQNLLYANSAHALDMTFEVDPMDEPTLLYVLFEVFDVVRVHQPHRGVIETVYLRT
PanAd2    ALTDLGQNLLYANSAHALDMTFEVDPMDEPTLLYVLFEVFDVVRVHQPHRGVIETVYLRT
PanAd3    ALTDLGQNLLYANSAHALDMTFEVDPMDEPTLLYVLFEVFDVVRVHQPHRGVIETVYLRT
ChAd55    ALTDLGQNMLYANSAHALDMNFEVDPMDESTLLYVVFEVFDVVRVHQPHRGVIEAVYLRT
ChAd73    ALTDLGQNMLYANSAHALDMNFEVDPMDESTLLYVVFEVFDVVRVHQPHRGVIKAVYLRT
ChAd83    ALTDLGQNMLYANSAHALDMNFEVDPMDESTLLYVVFEVFDVVRVHQPHRGVIEAVYLRT
ChAd146   ALTDLGQNMLYANSAHALDMNFEVDPMDESTLLYVVFEVFDVVRVHQPHRGVIEAVYLRT
ChAd147   ALTDLGQNMLYANSAHALDMNFEVDPMDESTLLYVVFEVFDVVRVHQPHRGVIEAVYLRT
          ********:***********.********.*****:******************::*****

PanAd1    PFSAGNATT
PanAd2    PFSAGNATT
PanAd3    PFSAGNATT
ChAd55    PFSAGNATT
ChAd73    PFSAGNATT
ChAd83    PFSAGNATT
ChAd146   PFSAGNATT
ChAd147   PFSAGNATT
          *********
```

Figure 2

Adenovirus Fiber Protein

CLUSTAL W (1.83) multiple sequence alignment

```
PanAd1    -MKRAKTSDETFNPVYPYDTENGPPSVPFLTPPFVSPDGFQESPPGVLSLRLSEPLVTSH
PanAd2    -MKRAKTSDETFNPVYPYDTENGPPSVPFLTPPFVSPDGFQESPPGVLSLRLSEPLVTSH
PanAd3    -MKRAKTSDETFNPVYPYDTENGPPSVPFLTPPFVSPDGFQESPPGVLSLRLSEPLVTSH
ChAd55    MSKKRVRVDDDFDPVYPYDADN-APTVPFINPPFVSSDGFQEKPLGVLSLRLADPVTTKN
ChAd73    MSKKRVRVDDDFDPVYPYDADN-APTVPFINPPFVSSDGFQEKPLGVLSLRLADPVTTKN
ChAd83    MSKKRVRVDDDFDPVYPYDADN-APTVPFINPPFVSSDGFQEKPLGVLSLRLADPVTTKN
ChAd146   MSKKRVRVDDDFDPVYPYDADN-APTVPFINPPFVSSDGFQEKPLGVLSLRLADPVTTKN
ChAd147   MSKKRARVDDGFDPVYPYDADN-APTVPFINPPFVSSDGFQEKPLGVLSLRLADPVTTKN
            *:    *: *:******::* .*:***:.*****.*****.* *******::*:.*.:

PanAd1    GMLALKMGNGLSLDDAGNLTSQDVTTVTPPLKKTKTNLSLQTSAPLTVSSGSLTVAAAAP
PanAd2    GMLALKMGNGLSLDDAGNLTSQDVTTVTPPLKKTKTNLSLQTSAPLTVSSGSLTVAAAAP
PanAd3    GMLALKMGNGLSLDDAGNLTSQDVTTVTPPLKKTKTNLSLQTSAPLTVSSGSLTVAAAAP
ChAd55    GEITLKLGEGVDLDDSGKLISKNAT----------------------------------
ChAd73    GEITLKLGEGVDLDDSGKLISKNAT----------------------------------
ChAd83    GEITLKLGEGVDLDSSGKLISNTAT----------------------------------
ChAd146   GEITLKLGEGLDLDSSGKLISNTAT----------------------------------
ChAd147   GAVPLKLGEGVDLDDSGKLISKKST----------------------------------
          * :.**:*:*:.**.:*:* *:  *

PanAd1    LAVAGTSLTMQSQAPLTVQDAKLGLATQGPLTVSEGKLTLQTSAPLTAADSSTLTVSATP
PanAd2    LAVAGTSLTMQSQAPLTVQDAKLGLATQGPLTVSEGKLTLQTSAPLTAADSSTLTVSATP
PanAd3    LAVAGTSLTMQSQAPLTVQDAKLGLATQGPLTVSEGKLTLQTSAPLTAADSSTLTVGTTP
ChAd55    ------------------------------------------------------KATA
ChAd73    ------------------------------------------------------KATA
ChAd83    ------------------------------------------------------KAAA
ChAd146   ------------------------------------------------------KAAA
ChAd147   ------------------------------------------------------KANS
                                                                    ..

PanAd1    PLSTSNGSLSIDMQAPIYTTNGKLALNIGAPLHVVD--TLNALTVVTGQGLTINGRALQT
PanAd2    PLSTSNGSLSIDMQAPIYTTNGKLALNIGAPLHVVD--TLNALTVVTGQGLTINGRALQT
PanAd3    PISVSSGSLGLDMEDPMYTHDGKLGIRIGGPLQVVD--SLHTLTVVTGNGITVANNALQT
ChAd55    PLSISNSTISLNMDAPLYNNNGKLGIRIGAPLKVVD--LLNTLAVAYGSGLGLKNNALTV
ChAd73    PLSISNSTISLNMAAPFYNNNGTLSLNVSTPLAVFP--TFNTLGISLGNGLQTSNKLLAV
ChAd83    PLSFSNNTISLNMDHPFYTKDGKLALQVSPPLNILRTSILNTLALGFGSGLGLRGSALAV
ChAd146   PLSFSNNTISLNMDHPFYTKDGKLSLQVSPPLNILRTSILNTLALGFGSGLGLRGSALAV
ChAd147   PLSISNNTISLNMDTPFYTKDGKLTMQVTAPLKLANTAILNTLAMAYGNGLGLNNNALTV
          *:* *..::.::*  *:*. :*.* :.:  ** :     :::* :  *.*:   .  * .

PanAd1    RVTGALSYDTEGNIQLQAGGG--------MRIDNNGQLILNVAYPFDAQNNLSLRLGQGP
PanAd2    RVTGALSYDTEGNIQLQAGGG--------MRIDNNGQLILNVAYPFDAQNNLSLRLGQGP
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PanAd3    KVAGALGYDSSGNLELRAAGG--------MRINTGGQLILDVAYPFDAQNNLSLRLGQGP
ChAd55    QLVSPLTFDNKGNVKINLGNGPLTVAANRLSVTCKRGLYVTTTG-DALESNISWAKG--I
ChAd73    QLTHPLTFSS-NSITVKTD----------------KGLYINSSGNRGLEANISLKRG--L
ChAd83    QLVSPLTFDTDGNIKLTLD----------------RGLHVTTG--DAIESNISWAKG--L
ChAd146   QLVSPLTFDTDGNIKLTLD----------------RGLHVTTG--DAIESNISWAKG--L
ChAd147   QVTSPLTFDN-SKVKINLGNGPLMVSANKLSINCLRGLYVAPNN-TGLETNISWANA--M
          ::. .* :.. ..: :                    * :       : *:*   .

PanAd1    LIVNSAHNLDLNLNRGLYLFTSGNTKKLEVNIKTAKGLFYDGTAIAINAGDGLQFGSGSD
PanAd2    LIVNSAHNLDLNLNRGLYLFTSGNTKKLEVNIKTAKGLFYDGTAIAINAGDGLQFGSGSD
PanAd3    LYVNTNHNLDLNCNRGLTTTTSSNTTKLETKIDS-------------------------
ChAd55    RFEGNAIAANIG--KGLEFGTTSSES----------------------------------
ChAd73    IFDGNAIATYLG--SGLDYGSYDSDGKTRPIITK--------------------------
ChAd83    KFEDGAIATNIG--NGLEFGSSSTET----------------------------------
ChAd146   KFEDGAIATNIG--NGLEFGSSSTET----------------------------------
ChAd147   RFEGNAMAVYIDTNKGLQFGTTSTET----------------------------------
             .      :.   **   : ..

PanAd1    TNPLQTKLGLGLEYDSNKAIITKLGTGLSFDNTGAITVGNKNDDKLTLWTTPDPSPNCRI
PanAd2    TNPLQTKLGLGLEYDSNKAIITKLGTGLSFDNTGAITVGNKNDDKLTLWTTPDPSPNCRI
PanAd3    ----------GLDYNANGAIIAKLGTGLTFDNTGAITVGNTGDDKLTLWTTPDPSPNCRI
ChAd55    ------------DVSNAYPIQVKLGTGLTFDSTGAIVAWNKEDDKLTLWTTADPSPNCHI
ChAd73    -------IGAGLNFDSNNAMAVKLGTGLSFDSAGALTAGNKEDDKLTLWTTPDPSPNCQL
ChAd83    ------------GVDDAYPIQVKLGSGLSFDSTGAIMAGNKEDDKLTLWTTPDPSPNCQI
ChAd146   ------------GVDDAYPIQVKLGSGLSFDSTGAIMAGNKEDDKLTLWTTPDPSPNCQI
ChAd147   ------------GVTNAYPIQVKLGAGLAFDSTGAIVAWNKENDSLTLWTTPDPSPNCKI
                     .: .***:**:**.:**: . *. :*.******.******::

PanAd1    NSEKDAKLTLVLTKCGSQVLASVSVLSVKG--SLAPISGTVTSAQIVLRFDENGVLLSNS
PanAd2    NSEKDAKLTLVLTKCGSQVLASVSVLSVKG--SLAPISGTVTSAQIVLRFDENGVLLSNS
PanAd3    HADKDCKFTLVLTKCGSQILASVAALAVSG--NLSSMTGTVSSVTIFLRFDQNGVLMENS
ChAd55    YSDKDAKLTLCLTKCGSQILGTVSLIAVDT-GSLNPITGQVTTALVSLKFDANGVLQTSS
ChAd73    LSDRDAKFTLCLTKCGSQILGTVAVAAVTVSSALNPINDTVKSAIVFLRFDSDGVLMSNS
ChAd83    LAENDAKLTLCLTKCGSQILATVSVLVVGS-GNLNPITGTVSSAQVFLRFDANGVLLTEH
ChAd146   LAENDAKLTLCLTKCGSQILATVSVLVVGS-GNLNPITGTVSSAQVFLRFDANGVLLTEH
ChAd147   ASEKDAKLTLCLTKCGSQILGTVSLLAVS--GSLAPITGAVSTALVSLKFNANGALLDKS
           ::.*.*:** *******:*.:*:   *     * .:.. *.:. : *:*: :*.*  .

PanAd1    SLDPQYWNYRKGDSTEGTAYTNAVGFMPNLTAYPKTQSQTAKSNIVSQVYLNGDKTKPMT
PanAd2    SLDPQYWNYRKGDSTEGTAYTNAVGFMPNLTAYPKTQSQTAKSNIVSQVYLNGDKTKPMT
PanAd3    SLDKEYWNFRNGNSTNATPYTNAVGFMPNLSAYPKTQSQTAKNNIVSEVYLHGDKSKPMI
ChAd55    TLDKEYWNFRKGDVTPAEPYTNAIGFMPNIKAYPKNTNSAAKSHIVGKVYLHGEVSKPLD
ChAd73    SMVGDYWNFREGQTTQSVAYTNAVGFMPNLGAYPKTQSKTPKNSIVSQVYLNGETTMPMT
ChAd83    STLKKYWGYRQGDSIDGTPYVNAVGFMPNLKAYPKSQSSTTKNNIVGQVYMNGDVSKPML
ChAd146   STLKKYWGYRQGDSIDGTPYTNAVGFMPNLKAYPKSQSSTTKNNIVGQVYMNGDVSKPML
ChAd147   TLNKEYWNYRQGDLIPGTPYTHAVGFMPNKKAYPKNTTAASKSHIVGDVYLDGDADKPLS
          :   .**.:*:*:   . .*.:*:***** ****. . :.*. **..**:.*:   *:

PanAd1    LTITLNGTNETG-DATVSTYSMSFSWNWNGS-NYINDTFQTNSFTFSYIAQE
PanAd2    LTITLNGTNETG-DATVSTYSMSFSWNWNGS-NYINDTFQTNSFTFSYIAQE
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PanAd3    LTITLNGTNESSETSQVSHYSMSFTWSWDSG-KYATETFATNSFTFSYIAEQ
ChAd55    LIITFNETSNE-----TCTYCINFQWQWGTD-KYKNETLAVSSFTFSYIAQE
ChAd73    LTITFNGTDEKD-TTPVSTYSMTFTWQWTGDYKDKNITFATNSFTFSYMAQE
ChAd83    LTITLNGTDDS-----NSTYSMSFSYTWTNG-SYVGATFGANSYTFSYIAQE
ChAd146   LTITLNGTDDS-----NSTYSMSFSYTWTNG-SYVGATFGANSYTFSYIAQE
ChAd147   LIITFNETDDE-----TCDYCINFQWKWGAD-QYKDKTLATSSFTFSYIAQE
          * **:* *.:       . *.:.* : *  . .    *: ..*:****:*::
```

Figure 3

Adenovirus Penton Protein

```
CLUSTAL W (1.83) multiple sequence alignment

PanAd1    -MRRAAMYHEGPPPSYESVVGA--AAASPFASQLEPPYVPPRYLRPTGGRNSIRYSELAP
PanAd2    -MRRAAMYHEGPPPSYESVVGA--AAASPFASQLEPPYVPPRYLRPTGGRNSIRYSELAP
PanAd3    -MRRAAMYHEGPPPSYESVVGA--AAASPFASQLEPPYVPPRYLRPTGGRNSIRYSELAP
ChAd55    MMRR--VYPEGPPPSYESVMQQ--AVAAAMQPPLEAPYVPPRYLAPTEGRNSIRYSELAP
ChAd73    MMRR--VYPEGPPPSYESVMQQ--AVAVAMQPPLEAPYVPPRYLAPTEGRNSIRYSELAP
ChAd83    MMRR--VYPEGPPPSYESVMQQ--AVAAAMQPPLEAPYVPPRYLAPTEGRNSIRYSELAP
ChAd146   MMRR--VYPEGPPPSYESVMQQ--AVAAAMQPPLEAPYVPPRYLAPTEGRNSIRYSELAP
ChAd147   MMRR--AYPEGPPPSYESVMQQAMAAAAAMQPPLEAPYVPPRYLAPTEGRNSIRYSELAP
           ***   * **********:    *.* .: . **.******** ** ************

PanAd1    LYDTTRVYLVDNKSADVASLNYQNDHSNFLTTVIQNNDYTPSEASTQTINLDDRSHWGGD
PanAd2    LYDTTRVYLVDNKSADVASLNYQNDHSNFLTTVIQNNDYTPSEASTQTINLDDRSHWGGD
PanAd3    LYDTTRVYLVDNKSADVASLNYQNDHSNFLTTVIQNNDYTPSEASTQTINLDDRSHWGGD
ChAd55    LYDTTRLYLVDNKSADIASLNYQNDHSNFLTTVVQNNDFTPTEASTQTINFDERSRWGGQ
ChAd73    LYDTTRLYLVDNKSADIASLNYQNDHSNFLTTVVQNNDFTPTEASTQTINFDERSRWGGQ
ChAd83    LYDTTRLYLVDNKSADIASLNYQNDHSNFLTTVVQNNDFTPTEASTQTINFDERSRWGGQ
ChAd146   LYDTTRLYLVDNKSADIASLNYQNDHSNFLTTVVQNNDFTPTEASTQTINFDERSRWGGQ
ChAd147   LYDTTRLYLVDNKSADIASLNYQNDHSNFLTTVVQNNDFTPTEASTQTINFDERSRWGGQ
          ******:*********:****************:****:**:********:*:**:***:

PanAd1    LKTILHTNMPNVNEFMFTNKFKARVMVSRSHTK---------DDRVELKYEWVEFELPEG
PanAd2    LKTILHTNMPNVNEFMFTNKFKARVMVSRSHTK---------DDRVELKYEWVEFELPEG
PanAd3    LKTILHTNMPNVNEFMFTNKFKARVMVSRSHTK---------DDRVELKYEWVEFELPEG
ChAd55    LKTIMHTNMPNVNEFMYSNKFKARVMVSRKTPNGVAVGDDYDGGQDELTYEWVEFELPEG
ChAd73    LKTIMHTNMPNVNEFMYSNKFKARVMVSRKTPNGVTVGDDYDGSQDELTYEWVEFELPEG
ChAd83    LKTIMHTNMPNVNEFMYSNKFKARVMVSRKTPNGVTVTD---GSQDELTYEWVEFELPEG
ChAd146   LKTIMHTNMPNVNEFLYSNKFKARVMVSRKTPNGVTVTD---GSQDELTYEWVEFELPEG
ChAd147   LKTIMHTNMPNVNEFMYSNKFKARVMVSRKTPNGVTVTEDYDGSQDELKYEWVEFELPEG
          ****:**********:::***********. .:         ..: **.***********

PanAd1    NYSETMTIDLMNNAIVEHYLKVGRQNGVLESDIGVKFDTRNFRLGLDPVTGLVMPGVYTN
PanAd2    NYSETMTIDLMNNAIVEHYLKVGRQNGVLESDIGVKFDTRNFRLGLDPVTGLVMPGVYTN
PanAd3    NYSETMTIDLMNNAIVEHYLKVGRQNGVLESDIGVKFDTRNFRLGLDPVTGLVMPGVYTN
ChAd55    NFSVTMTIDLMNNAIIDNYLAVGRQNGVLESDIGVKFDTRNFRLGWDPVTELVMPGVYTN
ChAd73    NFSVTMTIDLMNNAIIDNYLAVGRQNGVLESDIGVKFDTRNFRLGWDPVTELVMPGVYTN
ChAd83    NFSVTMTIDLMNNAIIDNYLAVGRQNGVLESDIGVKFDTRNFRLGWDPVTELVMPGVYTN
ChAd146   NFSVTMTIDLMNNAIIDNYLAVGRQNGVLESDIGVKFDTRNFRLGWDPVTELVMPGVYTN
ChAd147   NFSVTMTIDLMNNAIIDNYLAVGRQNGVLESDIGVKFDTRNFRLGWDPVTELVMPGVYTN
          *:* ***********:::** ************************ **** *********

PanAd1    EAFHPDIILLPGCGVDFTYSRLSNLLGIRKRQPFQEGFRITYEDLEGGNIPALLDVEAYQ
PanAd2    EAFHPDIILLPGCGVDFTYSRLSNLLGIRKRQPFQEGFRITYEDLEGGNIPALLDVEAYQ
```

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PanAd3    EAFHPDIILLPGCGVDFTYSRLSNLLGIRKRQPFQEGFRITYEDLEGGNIPALLDVEAYQ
ChAd55    EAFHPDIVLLPGCGVDFTESRLSNLLGIRKRQPFQEGFQILYEDLEGGNIPALLDVEAYE
ChAd73    EAFHPDIVLLPGCGVDFTESRLSNLLGIRKRQPFQEGFQILYEDLEGGNIPALLDVEAYE
ChAd83    EAFHPDIVLLPGCGVDFTESRLSNLLGIRKRQPFQEGFQILYEDLEGGNIPALLDVEAYE
ChAd146   EAFHPDIVLLPGCGVDFTESRLSNLLGIRKRQPFQEGFQIMYEDLEGGNIPALLDVEAYE
ChAd147   EAFHPDIVLLPGCGVDFTESRLSNLLGIRKRQPFQEGFQIMYEDLEGGNIPALLDVDAYE
          *******:********** ******************:* ***************:**:

PanAd1    DSLKEEEAGEGSGGG--AGQEEGGASSEASADPAAAAEAEAADPAMVVEEEKDMNDEAVR
PanAd2    NSLKEEEAGEGSGGGG-AGQEEGGASSEASADAAAAEAEEAADPAMVVEEEKDMNDEAVR
PanAd3    DSLKEEEAGEGSGGGGGAGQEEGGASSEASADAAAAAEAEAADPAMVVEEEKDMNDEAVR
ChAd55    KSKEE---------------------SAAAATAAVA-----------------TASTEVR
ChAd73    KSKEDS----------------AAATTAAVATAATTD----------------ADATTTR
ChAd83    KSKED---------------------STAVATAATV-----------------ADATVTR
ChAd146   KSKED---------------------SAAAATAAVA-----------------TASTEVR
ChAd147   KSKEE---------------------SAAAATAAVA-----------------TASTEVR
          .* ::                      : * * .*..                      .*

PanAd1    GDTFATRGEEKKAEAEAAAEEAAAAAAA-VEAAAEAEKPPKEPVIKPLTEDSKKRSYNVL
PanAd2    GDTFATRGEEKKAEAEAAAEEAAAAAAA-VEAAAEAEKPPKEPVIKPLTEDSKKRSYNVL
PanAd3    GDTFATRGEEKKAEAEAAAEEAAAAAAAAVEAAAEAEKPPKEPVIKALTEDSKKRSYNVL
ChAd55    GDNFASAAAVA----EAAETESKIVIQP-------------------VEKDSKDRSYNVL
ChAd73    GDTFATQAEEAAALAATDDSESKIVIKP-------------------VEKDSKDRSYNVL
ChAd83    GDTFATQAEEAAALAATDDSESKIVIKP-------------------VEKDSKDRSYNVL
ChAd146   GDNFASAAAVA----EAAETESKIVIQP-------------------VEKDSKDRSYNVL
ChAd147   GDNFASAAAVAA--AEAAETESKIVIQP-------------------VEKDSKDRSYNVL
          **.**: .       :   *:  .  .                    : : **.******

PanAd1    KDSTNTEYRSWYLAYNYGDPATGVRSWTLLCTPDVTCGSEQVYWSLPDMMQDPVTFRSTR
PanAd2    KDSTNTEYRSWYLAYNYGDPATGVRSWTLLCTPDVTCGSEQVYWSLPDMMQDPVTFRSTR
PanAd3    KDSTNTAYRSWYLAYNYGDPATGVRSWTLLCTPDVTCGSEQVYWSLPDMMQDPVTFRSTR
ChAd55    ADKKNTAYRSWYLAYNYGDPEKGVRSWTLLTTSDVTCGVEQVYWSLPDMMQDPVTFRSTR
ChAd73    ADKKNTAYRSWYLAYNYGDPEKGVRSWTLLTTSDVTCGVEQVYWSLPDMMQDPVTFRSTR
ChAd83    SDGKNTAYRSWYLAYNYGDPEKGVRSWTLLTTSDVTCGVEQVYWSLPDMMQDPVTFRSTR
ChAd146   ADKKNTAYRSWYLAYNYGDPEKGVRSWTLLTTSDVTCGVEQVYWSLPDMMQDPVTFRSTR
ChAd147   PDKINTAYRSWYLAYNYGDPEKGVRSWTLLTTSDVTCGVEQVYWSLPDMMQDPVTFRSTR
           *  ** ************* .******** *.***** **********************

PanAd1    QVSNFPVVGAELLPVHSKSFYNDQAVYSQLIRQFTSLTHVFNRFPENQILARPPAPTITT
PanAd2    QVSNFPVVGAELLPVHSKSFYNDQAVYSQLIRQFTSLTHVFNRFPENQILARPPAPTITT
PanAd3    QVSNFPVVGAELLPVHSKSFYNDQAVYSQLIRQFTSLTHVFNRFPENQILARPPAPTITT
ChAd55    QVSNYPVVGAELLPVYSKSFFNEQAVYSQQLRAFTSLTHVFNRFPENQILVRPPAPTITT
ChAd73    QVSNYPVVGAELLPVYSKSFFNEQAVYSQQLRAFTSLTHVFNRFPENQILVRPPAPTITT
ChAd83    QVSNYPVVGAELLPVYSKSFFNEQAVYSQQLRAFTSLTHVFNRFPENQILVRPPAPTITT
ChAd146   QVSNYPVVGAELLPVYSKSFFNEQAVYSQQLRAFTSLTHVFNRFPENQILVRPPAPTITT
ChAd147   QVSNYPVVGAELLPVYSKSFFNEQAVYSQQLRAFTSLTHVFNRFPENQILVRPPAPTITT
          ****:**********:****:*:****** :* *****************.*********


PanAd1    VSENVPALTDHGTLPLRNSIGGVQRVTVTDARRRTCPYVYKALGIVSPRVLSSRTF
PanAd2    VSENVPALTDHGTLPLRNSIGGVQRVTVTDARRRTCPYVYKALGIVSPRVLSSRTF
PanAd3    VSENVPALTDHGTLPLRNSIGGVQRVTVTDARRRTCPYVYKALGIVSPRVLSSRTF
ChAd55    VSENVPALTDHGTLPLRSSIRGVQRVTVTDARRRTCPYVYKALGVVAPRVLSSRTF
```

```
ChAd73    VSENVPALTDHGTLPLRSSIRGVQRVTVTDARRRTCPYVYKALGVVAPRVLSSRTF
ChAd83    VSENVPALTDHGTLPLRSSIRGVQRVTVTDARRRTCPYVYKALGVVAPRVLSSRTF
ChAd146   VSENVPALTDHGTLPLRSSIRGVQRVTVTDARRRTCPYVYKALGVVAPRVLSSRTF
ChAd147   VSENVPALTDHGTLPLRSSIRGVQRVTVTDARRRTCPYVYKALGIVAPRVLSSRTF
          *****************.** ************************:*:*********
```

Figure 4

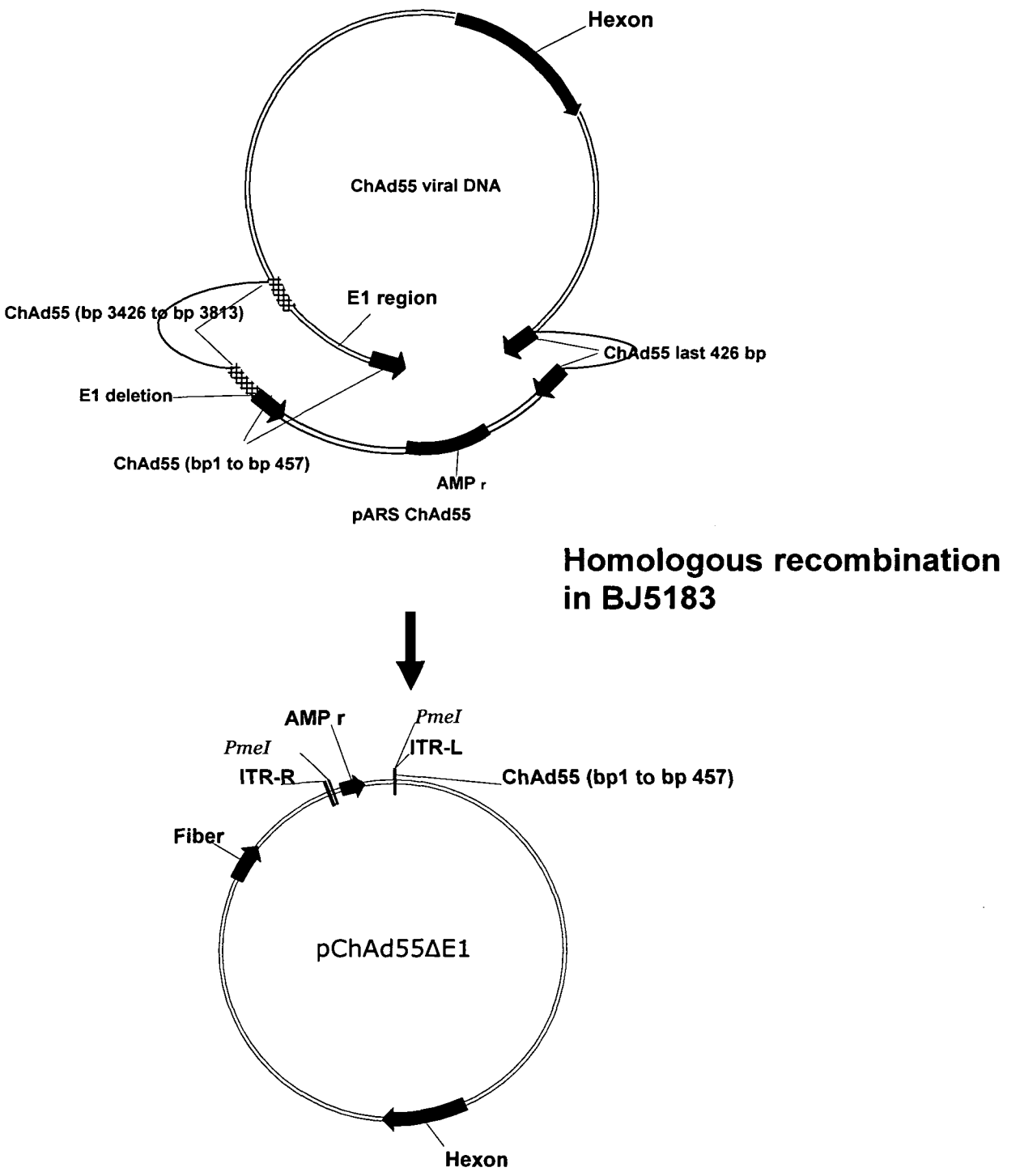

Hexon
ChAd55 viral DNA
E1 region
ChAd55 (bp 3426 to bp 3813)
ChAd55 last 426 bp
E1 deletion
ChAd55 (bp1 to bp 457)
AMP r
pARS ChAd55
Homologous recombination
in BJ5183
AMP r
PmeI
PmeI
ITR-L
ITR-R
ChAd55 (bp1 to bp 457)
Fiber
pChAd55ΔE1
Hexon

Figure 5A

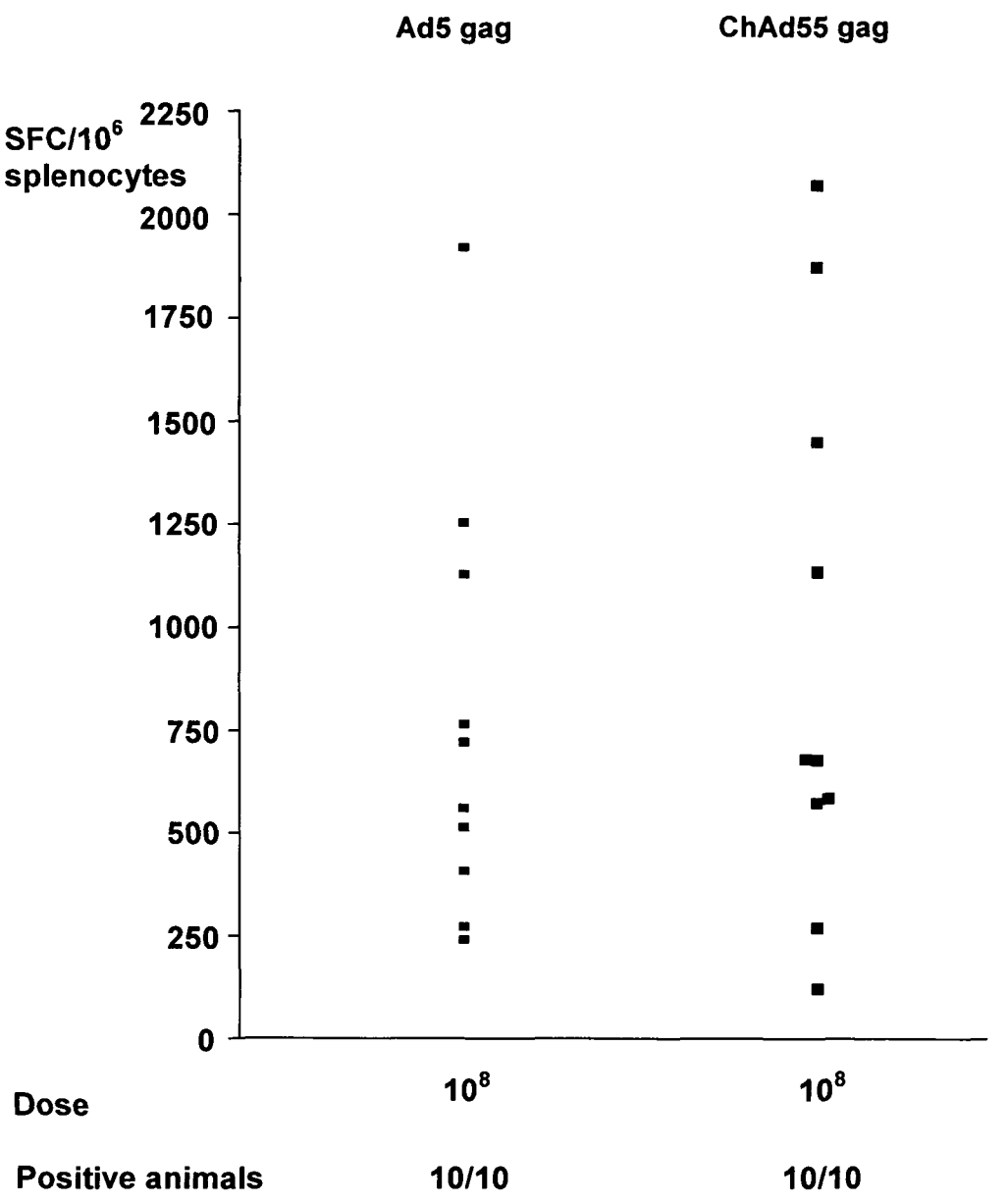

Ad5 gag
ChAd55 gag
SFC/10^6 splenocytes
2250
2000
1750
1500
1250
1000
750
500
250
0
Dose
10^8
10^8
Positive animals
10/10
10/10

Figure 5B

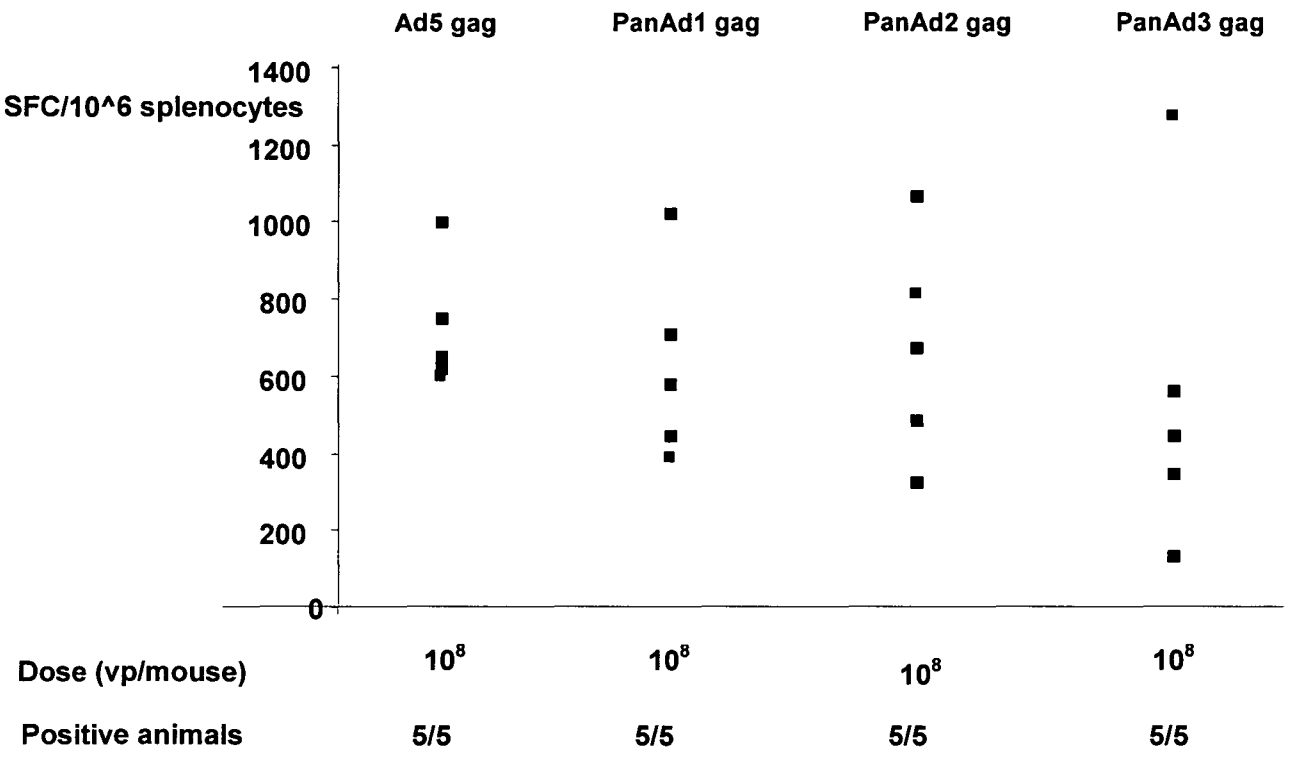


Figure 5C

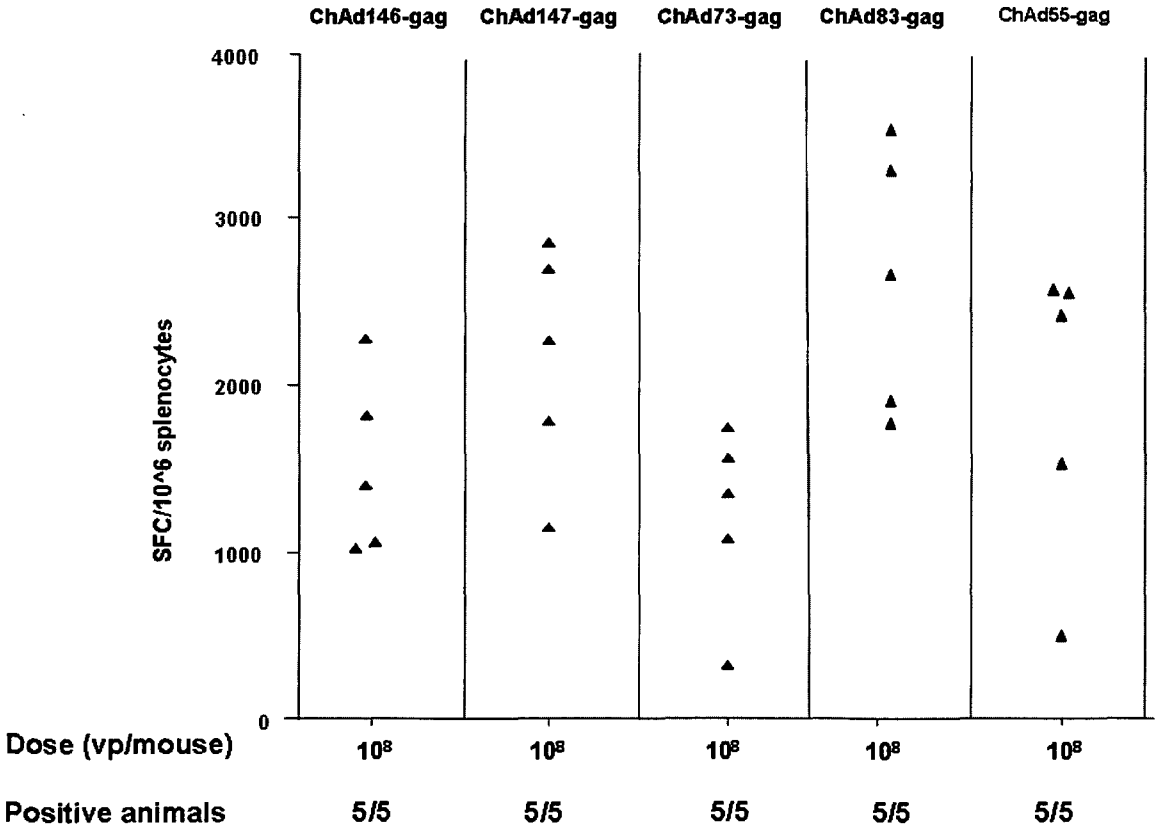


Figure 6

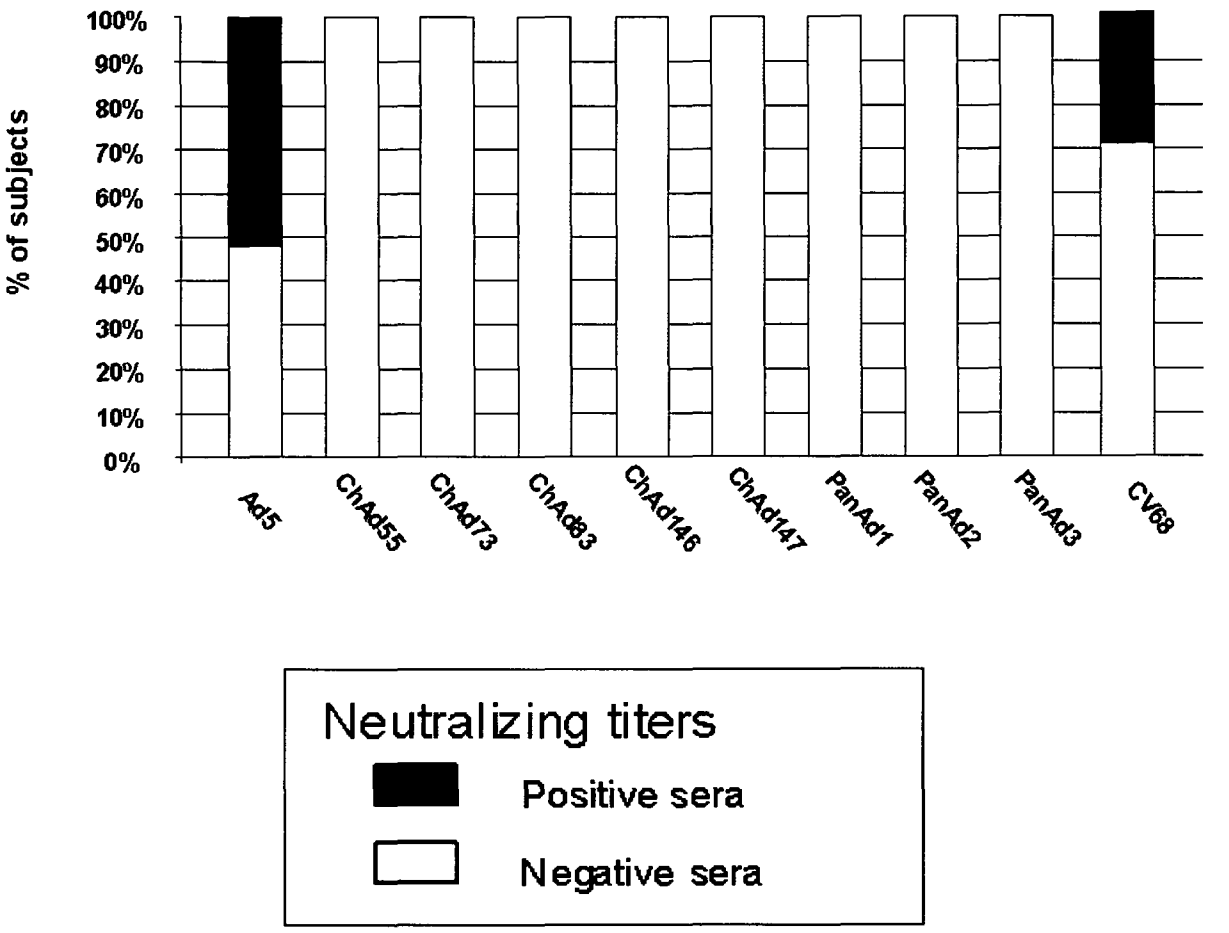

% of subjects
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Ad5
ChAd55
ChAd73
ChAd83
ChAd146
ChAd147
PanAd1
PanAd2
PanAd3
CV68
Neutralizing titers
Positive sera
Negative sera

Figure 7A

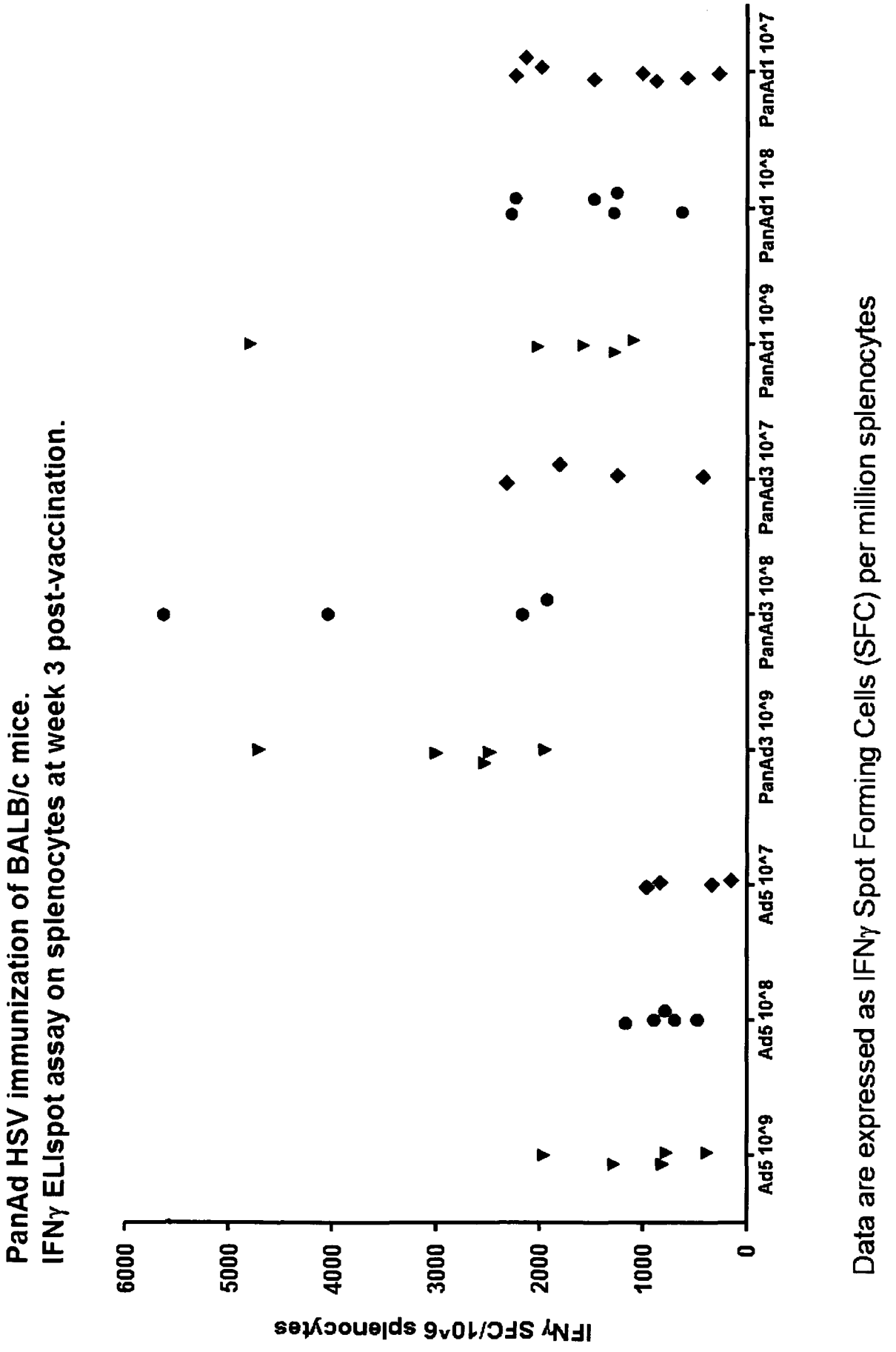

PanAd HSV immunization of BALB/c mice.
IFNγ ELIspot assay on splenocytes at week 3 post-vaccination.
IFNγ SFC/10^6 splenocytes
6000
5000
4000
3000
2000
1000
0
Ad5 10^9
Ad5 10^8
Ad5 10^7
PanAd3 10^9
PanAd3 10^8
PanAd3 10^7
PanAd1 10^9
PanAd1 10^8
PanAd1 10^7
Data are expressed as IFNγ Spot Forming Cells (SFC) per million splenocytes

Figure 7B

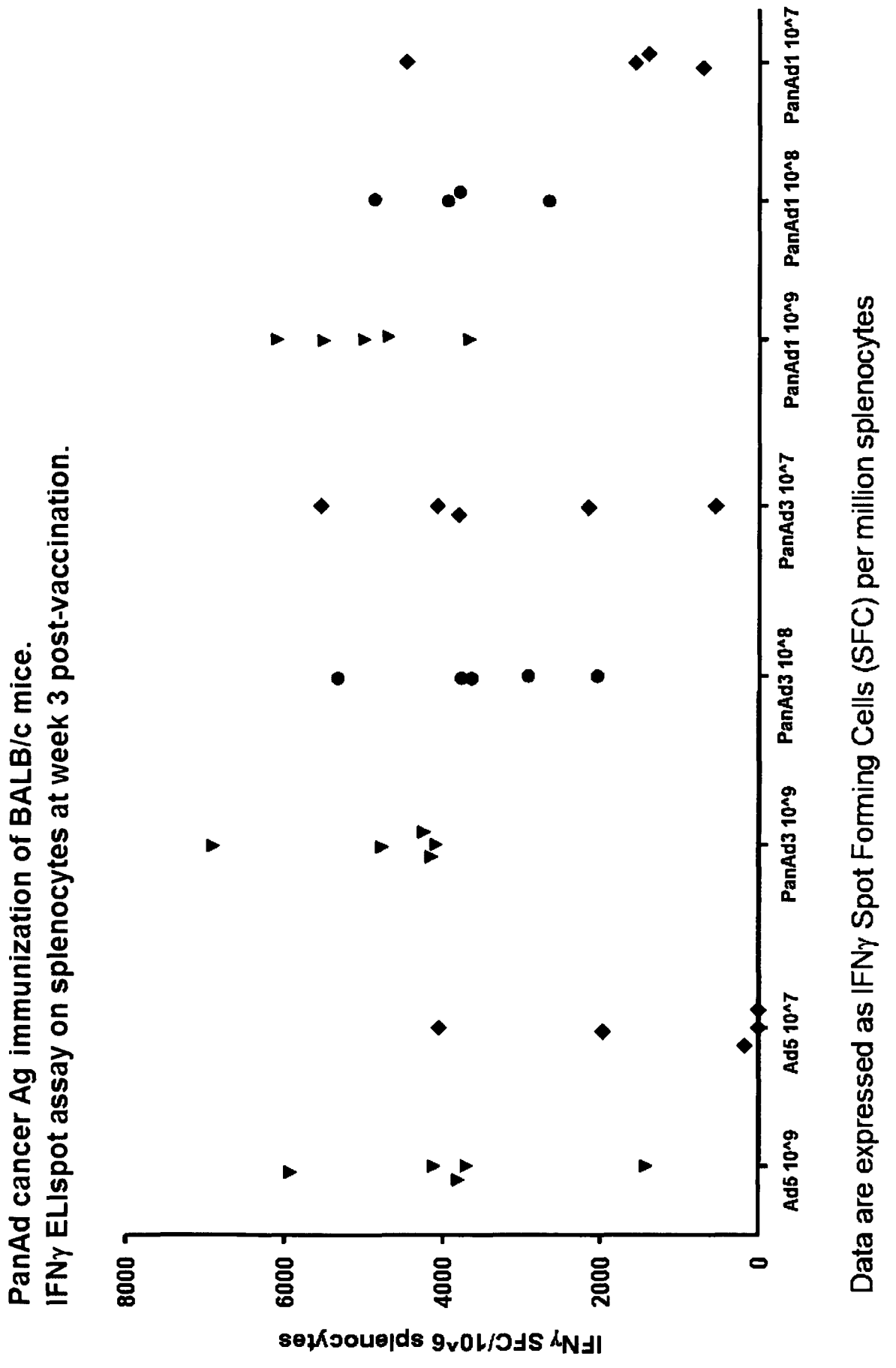
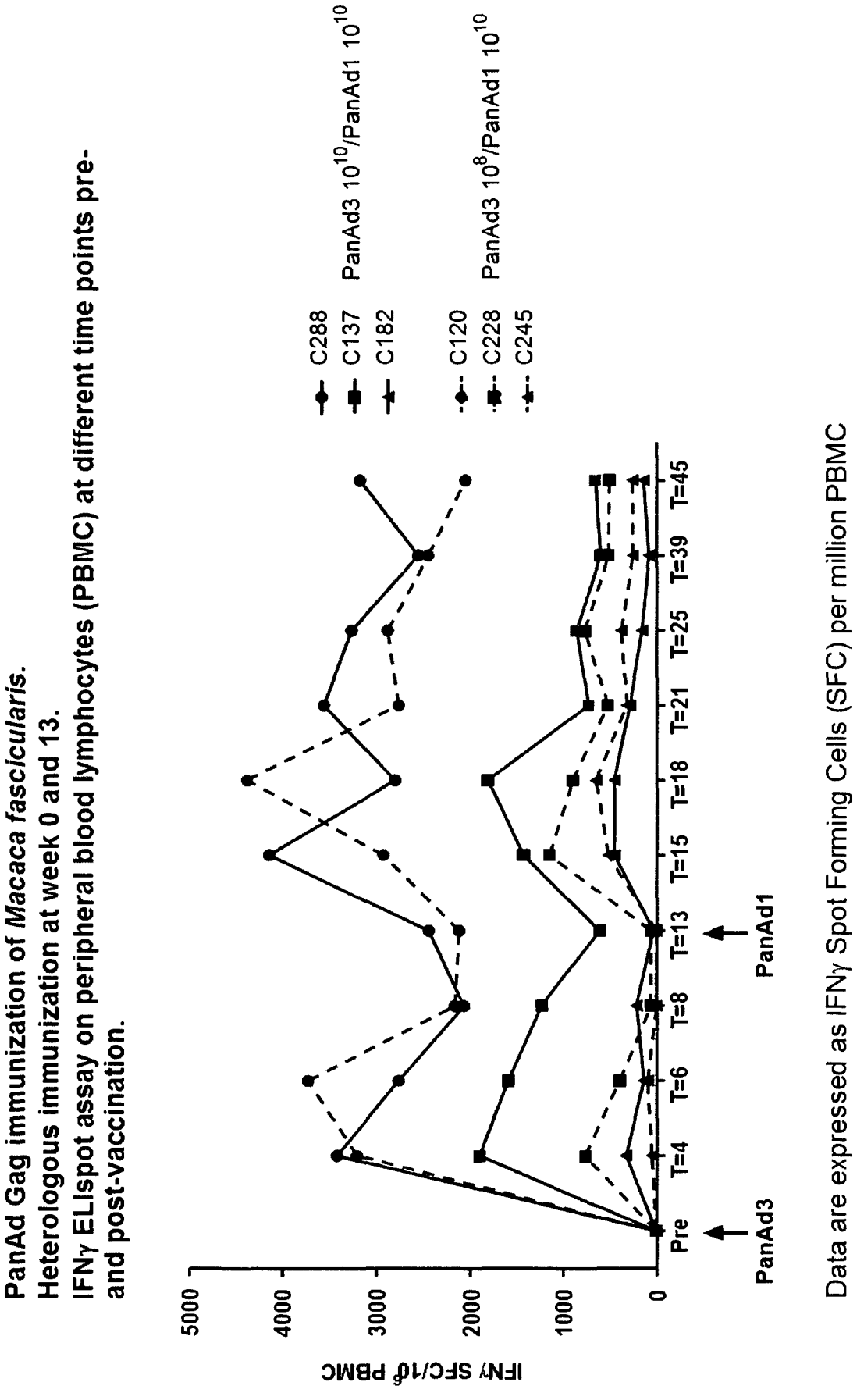

PanAd cancer Ag immunization of BALB/c mice.
IFNγ ELIspot assay on splenocytes at week 3 post-vaccination.
IFNγ SFC/10^6 splenocytes
8000
6000
4000
2000
0
Ad5 10^9
Ad5 10^7
PanAd3 10^9
PanAd3 10^8
PanAd3 10^7
PanAd1 10^9
PanAd1 10^8
PanAd1 10^7
Data are expressed as IFNγ Spot Forming Cells (SFC) per million splenocytes

Figure 8


PanAd Gag immunization of Macaca fascicularis.
Heterologous immunization at week 0 and 13.
IFNγ ELIspot assay on peripheral blood lymphocytes (PBMC) at different time points pre- and post-vaccination.
C288
C137
C182
PanAd3 10^10/PanAd1 10^10
C120
C228
C245
PanAd3 10^8/PanAd1 10^10
IFNγ SFC/10^6 PBMC
5000
4000
3000
2000
1000
0
Pre
T=4
T=6
T=8
T=13
T=15
T=18
T=21
T=25
T=39
T=45
PanAd3
PanAd1
Data are expressed as IFNγ Spot Forming Cells (SFC) per million PBMC