In relation to adenovirus, the present inventors found that the inclusion of the peptide pairs within the adenoviral capsid proteins were not as routine or straightforward as postulated, particularly in relation to the provision of an attachment means for antigens. Indeed, work by the inventors has shown that the most commonly utilised peptide pair, in this case insertion of SpyTag into the hexon capsid protein did not allow for the formation of a functional adenoviral vector (a vector allowing for effective coupling of SpyCatcher and capable of infecting cells). Indeed, the data showing that SpyTag was poorly reactive after insertion is included in the Examples, together with data on the lack of infectivity of the modified adenovirus. Thus, significant work has been expended in developing a system that works in relation to a modified adenoviral vector that is ready to accept peptide pairing and attachment of an entity to the modified capsid protein via the peptide partner inserted into the capsid protein.

Summary ~~of the Invention~~

In an aspect of the present disclosure, there is provided an adenoviral vector for preparation of a prophylactic or therapeutic composition, comprising at least one modification in a capsid protein, wherein said modification comprises the inclusion of a first peptide partner in a capsid protein, and said first peptide is capable of forming a covalent bond with a second peptide partner.

In another aspect of the present disclosure, there is provided an adenoviral vector disclosed herein for use in the manufacture of a vaccine comprising the addition of a second peptide partner attached to an antigen to said adenoviral vector.

In a further aspect of the present disclosure, there is provided a vaccine comprising the adenoviral vector as disclosed herein, wherein an antigen is attached to the adenoviral vector via the second peptide partner.

In another aspect of the present disclosure, there is provided a method of producing an adenoviral vector as disclosed herein, comprising:

- introducing a nucleic acid which encodes a first peptide partner into the nucleic acid encoding a capsid protein;
- inserting the modified capsid gene into the genome of an appropriate adenovirus; and infecting a cell with said adenovirus and collecting the released progeny virus.

In a further aspect of the present disclosure, there is provided a vaccine comprising an adenoviral vector as disclosed herein and an attached antigen for use in the treatment or prevention of a disease.

In another aspect of the present disclosure, there is provided a kit comprising an adenoviral vector as disclosed herein.

In a further aspect of the present disclosure, there is provided a vaccine comprising an adenoviral vector as disclosed herein, wherein

a. said first peptide partner is SpyCatcher which is fused to the pIX capsid protein, and said second peptide partner is SpyTag, which is attached to an antigen, and wherein SpyCatcher and SpyTag are bound covalently through an isopeptide bond;
b. said first peptide partner is SnoopCatcher which is fused to the pIX capsid protein, and said second peptide partner is SnoopTagJr, or SnoopTag which is attached to an antigen, and wherein SnoopCatcher and SnoopTagJr or SnoopTag are bound covalently through an isopeptide bond; or
c. said first peptide partner is DogCatcher which is fused to the pIX capsid protein, and said second peptide partner is DogTag which is attached to an antigen, and wherein DogCatcher and DogTag are bound covalently through an isopeptide bond; or
d. said first peptide partner is SnoopTagJr which is fused to the pIX capsid protein, and said second peptide partner is SnoopCatcher, which is attached to an antigen, and wherein SnoopTagJr and SnoopCatcher are bound covalently through an isopeptide bond;

said first peptide partner is SpyTag which is fused to the pIX capsid protein, and said second peptide partner is SpyCatcher, which is attached to an antigen, and wherein SpyTag and SpyCatcher are bound covalently through an isopeptide bond.

In another aspect of the present disclosure, there is provided a vaccine comprising an adenoviral vector as disclosed herein, wherein said first peptide partner is DogTag which is inserted into HVR loops of the hexon protein, and said second peptide partner is DogCatcher or SnoopTagJr, said second peptide partner being attached to an antigen, and wherein DogTag and DogCatcher/SnoopTagJr are bound covalently through an isopeptide bond.

In a further aspect of the present disclosure, there is provided an oncolytic virus preparation comprising an adenoviral vector as disclosed herein, wherein said second peptide partner is attached to a shielding entity or a targeting moiety.

In another aspect of the present disclosure, there is provided an adenoviral vector capable of surface decoration via an isopeptide linkage via a modified capsid protein.

In accordance with a first ~~aspect~~example of the ~~invention~~present disclosure, there is provided an adenoviral vector for preparation of a prophylactic or therapeutic adenoviral composition, comprising at least one modification in the capsid protein, wherein said modification comprises the inclusion of a first peptide partner in a capsid protein, wherein said first peptide is capable of forming a covalent bond with a second peptide partner, which can be attached to an entity.

The modification may be a fusion with the first peptide partner or an insertion of the first peptide partner into the capsid protein.

The capsid protein may be any capsid protein, but is preferably a hexon protein or a pIX protein.

The first peptide partner and the second peptide partner form a peptide partner pair, which may be covalently linked by an isopeptide or ester bond, preferably an isopeptide bond.

In one ~~aspect~~example, the first peptide partner is the “tag” partner, which may be covalently linked by an isopeptide or ester bond to a second peptide partner which is a “catcher”. In this ~~aspect~~example, the capsid protein is preferably a hexon protein. Thus, the first peptide partner or “tag” which modifies the hexon is preferably a DogTag, Isopeptag, Isopeptag-N, SdyTag, PsCsTag or Jo. It is preferred that the first peptide partner or tag is not SpyTag. In this ~~embodiment~~example, SpyTag is unmodified during its insertion into the hexon protein.

In an alternative ~~aspect~~example, the first peptide partner is the “catcher” partner, which may be covalently linked by an isopeptide or ester bond to a second peptide partner which is a “tag”. In this ~~aspect~~example, the capsid protein is preferably a hexon protein. Thus, the first peptide partner or “catcher” which modifies the hexon may be a DogCatcher, SpyCatcher, SnoopCatcher, Pilin-C, Pilin-N, SdyCatcher, PsCsCatcher or In.

In one ~~aspect~~example, the first peptide partner is the “catcher” partner, which may be covalently linked by an isopeptide or ester bond to a second peptide partner which is a “tag”. In this ~~aspect~~example, the capsid protein is preferably a pIX protein. Thus, the first peptide partner or “catcher” which modifies the pIX is preferably a SpyCatcher, DogCatcher, SnoopCatcher, Pilin-C, Pilin-N, SdyCatcher, PsCsCatcher or In.

In an alternative ~~aspect~~example, the first peptide partner is the “tag” partner, which may be covalently linked by an isopeptide or ester bond to a second peptide partner which is a “catcher”. In this ~~aspect~~example, the capsid protein is preferably a pIX protein. Thus, the first peptide partner or “tag” which modifies the pIX protein is preferably a SpyTag, SnoopTagJr, DogTag, Isopeptag, Isopeptag-N, SdyTag, PsCsTag or Jo.

In one ~~aspect~~example, the first peptide partner may be inserted into a hexon protein, and optionally the insertion into the hexon protein may be up to 200, up to 150 or up to 100 amino acids in length. The insertion into the hexon protein may be at any appropriate point, optionally in any one or more of the hypervariable HVR loops. The first peptide partner inserted into the hexon protein may be a DogTag. DogTag is capable of forming a spontaneous covalent bond with DogCatcher, or a covalent bond with SnoopTagJr or SnoopTag in a reaction requiring a catalyst, SnoopLigase. DogCatcher or SnoopTagJr or SnoopTag may therefore be the second peptide partner. DogTag or SnoopTagJr may therefore be the second peptide partner. The second peptide partner is linked or attached to an entity such as an antigen. It is surprising to the inventors that DogTag was able to be inserted into the hexon capsid protein to form a functional adenovirus vector for capsid display of protein partners after the failure of the SpyTag insertion, as described above. As the Examples show, the insertion of DogTag into the hexon and pairing with SnoopTagJr or DogCatcher enables the modified adenovirus to retain its infective ability in the cells tested after coupling. A wide range of SnoopTagJr and DogCatcher fusion proteins, some over 50 kDa in size, have been coupled efficiently

and displayed on adenovirus with no reduction in viral infectivity. The retention of infectivity is important if the therapeutic use of the adenoviral vector requires cell entry, for example for gene therapy or as an oncolytic virus.

Other first peptide partners that may be included within the hexon protein that are less than 100 amino acids in length include:

Isopeptag which pairs with Pilin-C

Isopeptag-N which pairs with Pilin-N

SdyTag which pairs with SdyCatcher

PsCsTag which pairs with PsCsCatcher

Jo which pairs with In, or

RrgATag/RrgATag2/DogTag which pair with RrgACatcher (also denoted here as "DogCatcher").

It is preferred that of the possible peptide partners that are included within the hexon protein, the inclusion is not an insertion of SpyTag. In this ~~embodiment~~example, SpyTag was inserted within various HVRs of hexon and found to reduce infectivity to an unsatisfactory level once paired with SpyCatcher. Thus, viral fitness was impaired.

In another ~~aspect~~example, the first peptide partner may be fused to the pIX capsid protein, optionally at the N- or C- terminal end, preferably at the C-terminal end. The first peptide partner fused to the pIX capsid protein may be a SpyCatcher, SnoopCatcher or DogCatcher. SpyCatcher is capable of forming a covalent bond with SpyTag, which herein forms the second peptide partner, and can therefore be attached to an antigen. SnoopCatcher is capable of forming a covalent bond with either SnoopTag or SnoopTagJr, and DogCatcher is capable of forming a covalent bond with DogTag, and may be used as a binding pair in either orientation as first or second peptide partner. The first peptide partner may also be a DogTag, SpyTag, SnoopTagJr or SnoopTag, wherein the matching second peptide partner is DogCatcher, SnoopTagJr, SnoopTag, SpyCatcher, DogTag, or SnoopCatcher. Other peptide partner pairs that may be suitable for fusion with pIX in either orientation are: RrgATag/RrgATag2/DogTag and RrgACatcher, Isopeptag/Pilin-C, Isopeptag-N/Pilin-N, SdyTag/SdyCatcher, PsCsTag/PsCsCatcher and Jo/In.

The insertion of SpyCatcher into pIX has been demonstrated to permit the coupling of SpyTag conjugated entities, including the HCMV pentamer, whilst retaining infectivity.

Particularly preferred may be the insertion of SnoopCatcher or DogCatcher into pIX, since these have both been demonstrated herein to have good adenoviral viability. These insertions are furthermore genetically stable for greater than 3 passages.

As used herein for any ~~aspect~~example of the present disclosure~~invention~~, the first peptide partner is part of a pair of peptides that are capable of forming a covalent bond, such as an isopeptide bond or ester bond under the appropriate conditions. These are also known as protein tag and catcher pairs or protein tag and binding partner pairs. The first peptide partner may be a first peptide tag or may be a first peptide catcher. Each partner pair may comprise a tag and a catcher. The covalent bond that is formed may spontaneously react, or require the assistance of a third entity such as a ligase. Further information on suitable peptide pairs is included below.

Said adenoviral vector may be used in the preparation of a vaccine. The vaccine may be prophylactic or therapeutic. The present disclosure~~invention~~ therefore extends to a method of preparing a vaccine, comprising the use of an adenoviral vector as described herein. The method comprises the attachment of an antigen to the adenoviral vector via a second peptide partner. Said second peptide partner is attached to the antigen, preferably fused to said antigen, and is capable of forming a covalent bond with the first peptide partner present on the immunogenic adenoviral vector. The covalent bond, and therefore attachment, may occur spontaneously, or may require the use of a third entity to facilitate binding, such as a ligase. Thus, the antigen is attached to the adenovirus by means of the peptide partner pair, the first partner of which is included within a modified capsid protein.

In another ~~aspect~~example of the present disclosure~~invention~~, there is provided a vaccine composition comprising an adenoviral vector including at least one modification in a capsid protein, wherein said modification comprises the inclusion of a first peptide partner, and said first peptide partner is covalently bonded to a second peptide partner attached to an antigen. Said adenoviral vector is as described extensively herein.

In another ~~aspect~~example of the present disclosure~~invention~~, there is provided an immunogenic adenoviral vector comprising of at least one modification in the hexon capsid protein, wherein said modification comprises:

a. a first peptide partner; and

b. a second peptide partner attached to an antigen

wherein the first peptide partner and second peptide partner are coupled via a covalent bond.

In one ~~embodiment~~example of this ~~aspect~~example of the present disclosure~~invention~~, the first peptide partner inserted into the hexon capsid protein is less than 200 amino acids in length, less than 150 amino acids in length, less than 100 amino acids in length, optionally wherein said first peptide partner is a DogTag. If DogTag is the first peptide partner, the second peptide partner may be DogCatcher or SnoopTagJr or SnoopTag. In order to assist the formation of a covalent bond between DogTag and SnoopTagJr or SnoopTag, a ligase may be utilised.

The first peptide partner inserted into the hexon may also be any one of SnoopTag, SnoopTagJr, SnoopCatcher, DogCatcher, Isopeptag, Pilin-C, Isopeptag-N, Pilin-N, SdyTag, SdyCatcher, PsCsTag, PsCsCatcher, RrgATag/RrgATag2, RrgACatcher, Jo, or In.

It is preferred that SpyTag is not used as the first peptide partner in the hexon protein, with SpyCatcher as the second peptide partner.

In another ~~aspect~~example of the present disclosure~~invention~~, there is provided an immunogenic adenoviral vector comprising of at least one modification in the pIX capsid protein, wherein said modification comprises:

a. a first peptide partner; and

b. a second peptide partner attached to an antigen

wherein the first peptide partner and second peptide partner are coupled via a covalent bond.

According to one ~~aspect~~example, the first peptide partner may be a SpyCatcher protein. In this instance, the second peptide partner may be a SpyTag. The covalent bond that forms between these partner pairs is spontaneous and does not require assistance. SnoopCatcher or DogCatcher could equally be utilised as the first peptide partner, with their partner pairs being SnoopTag/SnoopTagJr or DogTag respectively. SnoopTagJr/SnoopTag or SpyTag could equally be utilised as the first peptide partner, with their partner pairs being SnoopCatcher or SpyCatcher respectively.

adenoviral vector. The different entities may be for the same indication (for example different antigens for the same disease) or different indications (for example a combined vaccine composition for different diseases such as measles, mumps and rubella on a single adenoviral vector).

It is not generally an object of the present disclosure~~invention~~ in relation to vaccines to be altering the natural tropism of the adenovirus, since the present ~~invention~~ disclosure is seeking to raise antibodies or T cells to the presented antigen, rather than to retarget the vector to specific cell types. The fiber component is thought to be the major determinant of tropism. The inventors have seen for several Examples using a DogTag modification in the hexon protein that the modification to the capsid protein has little or no effect on the capability of transducing a specific target cell. Indeed, in the Examples, there was no drop in infectivity in HEK293 cells when DogTag was inserted into the hexon and paired with DogCatcher on the antigen. However, in some instances, the inventors have shown that modifications can affect the ability of the virus to infect HEK293 cells, herein this was demonstrated with SpyTag when inserted into the hexon and paired with SpyCatcher on the antigen. A 100-fold decrease in infectivity was seen for the SpyTag-inserted adenovirus. This is undesirable and unexpected. Indeed, given that this is the most commonly used peptide tag, and is frequently preferred for most applications, the inventors were of the opinion that adenovirus may be intransigent to the modification of the capsid protein using partner pairs. It was only through significant work that it was appreciated that this did not hold true for all partner pairs, and that DogTag in particular was a workable modification of the hexon protein.

The modified adenoviral vectors described here have been carefully engineered such that they are readily produced with yields comparable to vectors with a wild-type capsid protein, e.g. wild-type hexon or wild-type pIX capsid proteins. Methods of producing adenovirus *in vitro* are well known to those skilled in the art, along with methods of introducing recombinant genes into the adenovirus.

It is widely appreciated in the field that performing modifications and / or insertions to viral capsid proteins is difficult and often disruptive to viral function and replication. Modification must not inhibit correct folding of the capsid protein or its incorporation within the capsid structure of the virion during assembly. For antigen display in particular, modifications must be performed such that the conformation and location of the displayed antigen enables recognition by the immune system, and in the case of infectious viral particles, does not impede the ability of the viral vector to infect

cells. Previous studies have demonstrated the complexity of these requirements. In the context of hexon HVR loops, Matthews *et al* showed that vector viability is dependent upon the size of insertion and which HVR loop is targeted. [Matthews QL, *et al* Optimization of capsid-incorporated antigens for a novel adenovirus vaccine approach. Virol J. 2008 Aug 21;5:98. doi: 10.1186]. The same study also demonstrated that immune recognition by displayed epitopes was also dependent on the nature and location of the inserted antigen. Other studies, such as in Gu *et al*, have revealed the importance of the nature and length of linker sequences to facilitate efficient epitope display. [Linlin Gu, *et al*, A recombinant adenovirus-based vector elicits a specific humoral immune response against the V3 loop of HIV-1 gp120 in mice through the "Antigen Capsid-Incorporation" strategy, Virol J. 2014 Jun 16;11:112. doi: 10.1186]. In the present application, the present inventors again demonstrate this phenomenon, by showing that coupling of SpyCatcher to Ad5-HVR-SpyTag vectors is less efficient, and substantially impedes vector infectivity (Figure 2), while coupling of Ad5-HVR-DogTag to DogCatcher is highly efficient and does not impede vector infectivity (Figure 3). The superior efficiency and utility of DogTag:DogCatcher coupling compared to SpyTag:SpyCatcher coupling in the context of adenovirus hexon insertion was unexpected, since SpyTag:SpyCatcher coupling has been shown to be highly efficient in a wide variety of other contexts. Indeed, there are over 500 documented constructs using the SpyCatcher:SpyTag technology.

In the context of pIX modification, studies have demonstrated that the pIX protein can tolerate a variety of different extensions to the C-terminus. However, the viability, capsid stability, and efficiency of antigen display are highly dependent on the nature and length of the fusion [Igor P. Dmitriev, *et al*, Engineering of Adenovirus Vectors Containing Heterologous Peptide Sequences in the C Terminus of Capsid Protein IX. J Virol. 2002 Jul;76(14):6893-9] [Nadine C. Salisch, *et al*, Antigen capsid-display on human adenovirus 35 via pIX fusion is a potent vaccine platform. PLoS One. 2017; 12(3): e0174728].

In some ~~embodiment~~examples of any ~~aspect~~example of the present disclosure~~invention~~ at least one modification in the capsid protein of the virus is within a major capsid protein or within a minor capsid protein. All of the adenovirus proteins on the outer surface of the virion (hexon, fiber, protein IX and penton) are capable of being modified.

In one ~~embodiment~~example, the modification in the major capsid protein is in the hexon protein.

In other ~~embodiment~~examples, the modification in the minor capsid protein is in the pIX protein.

For any ~~aspect~~example of the present disclosure~~invention~~, at least one modification is the insertion or fusion of a first peptide partner to the capsid protein. Preferably, at least one modification is the insertion or fusion of a first peptide partner to the capsid protein via genetic modification of the adenovirus genome. Suitably, the modification is the insertion of a first peptide partner in the hexon protein, preferably into one or more of the HVR loops of the hexon protein. Thus, the insertion may be made into the sequence of the hexon protein, rather than fusing the first peptide partner to the N- or C- terminus. Suitably, the modification is the fusion of a first peptide partner in the pIX protein. For the pIX fusion, the first peptide partner may be fused at any suitable location, optionally the C- or N- terminal ends, preferably the C-terminal end. Those skilled in the art of modifying an adenovirus would be aware of how to introduce genetic modifications in order to modify the viral coat proteins as discussed herein.

In some ~~embodiment~~examples of any ~~aspect~~example of the present disclosure~~invention~~ a covalent bond is an isopeptide. This allows irreversible conjugation of the first peptide partner to the second peptide partner forming covalently stabilised multi-protein complexes. This isopeptide bond may be spontaneous, i.e. without assistance, or require assistance, i.e. from a ligase. Specific peptide partner pairs are well known in the art, and includes the non-exhaustive list: SpyTag/SpyCatcher and derivatives and modifications thereof, SnoopTag/SnoopCatcher, DogTag/DogCatcher, SnoopTagJr/SnoopCatcher, SnoopTagJr/Dog Tag, Isopeptag/Pilin-C, Isopeptag-N/Pilin-N, SdyTag/SdyCatcher, PsCsTag/PsCsCatcher, RrgATagor RrgATag2/RrgACatcher and/or Jo/In and modifications and variants of any of these partner pairs.

In one ~~embodiment~~example, a first peptide partner may be DogTag, or SpyCatcher. Alternatively, a first peptide partner may be SpyTag. The adenoviral capsid protein inserted with DogTag is assigned the term “Ad-DogTag”. The adenoviral capsid protein inserted with SpyTag is assigned the term “Ad-SpyTag”. The adenoviral capsid protein fused with SpyCatcher is assigned the term “Ad-SpyCatcher”.

In other ~~embodiment~~examples, a second peptide partner may be DogCatcher, SnoopTagJr, or SpyTag. In one example, a first peptide partner couples to a second peptide partner forming an isopeptide bond to make a peptide: peptide binding pair.

In one ~~embodiment~~example, a first peptide partner is DogTag and a second peptide partner is DogCatcher. The attachment of a first peptide partner for example, DogTag attached to the hexon capsid protein forms the vector Ad-DogTag. Upon coupling to the second peptide partner DogCatcher linked to an antigen, the vaccine vector formed is called Ad-DogTag:DogCatcher-Antigen. In another ~~embodiment~~example, a first peptide partner is DogTag and a second peptide partner is SnoopTagJr or SnoopTag. The synthesis of SnoopTagJr with an antigen such as a neoepitope results in SnoopTagJr fusion. The synthesis of SnoopTag with an antigen such as a neoepitope results in SnoopTag fusion. The coupling of either SnoopTagJr or SnoopTag fusion to the vector Ad-DogTag results in the vector called Ad-DogTag:SnoopTagJr-fusion or Ad-DogTag:SnoopTag-fusion, respectively. This reaction requires SnoopLigase to facilitate the coupling between DogTag and SnoopTagJr fusion, or SnoopTag fusion. It may be preferred that DogTag is attached to the hexon capsid protein, optionally to a HVR loop. In some ~~embodiment~~examples, the DogTag may be used to modify more than one HVR loop within the same hexon protein. This may increase the opportunity for attachment to the viral capsid.

In another ~~embodiment~~example, a first peptide partner is SpyCatcher and a second peptide partner is SpyTag. The SpyTag is linked to an antigen to form Ad-SpyCatcher:SpyTag-Antigen. Thus, it can be seen that the first peptide partner can be either what is termed in the art as a “tag” or “catcher”; with the second peptide partner component being the partner for this pair, the “catcher” or the “tag”, respectively. Herein both are designated as protein partners as they may be provided in either orientation, modifying the capsid protein or attached to the antigen. The coupling of the first peptide partner to the second peptide partner enables display of the antigens on the surface of the immunogenic adenoviral viral proteins. In this ~~embodiment~~example, the capsid protein is preferably pIX. Other “first peptide partner” and “second peptide partner” combinations that may be used in the context of pIX are primary partner SnoopTagJr and secondary partner SnoopCatcher, primary partner SpyTag and secondary partner SpyCatcher, primary partner SnoopCatcher and secondary partner SnoopTagJr or primary partner DogCatcher and secondary partner DogTag.

In another ~~embodiment~~example, the first peptide partner inserted into pIX may be any one of SnoopCatcher, DogCatcher, SpyTag and SnoopTagJr. These have all been demonstrated to be effective insertions, with good yield viability and are accessible for their respective second binding pair to allow decoration of the adenovirus with a desired entity. The respective second peptide partners are discussed here throughout. The resultant adenoviruses may be termed “Ad –

SnoopCatcher", "Ad-DogCatcher", "Ad-SpyTag" and "Ad-SnoopTagJr". Alternatively, these may be named "Ad pIX – SnoopCatcher", "Ad pIX -DogCatcher", "Ad pIX -SpyTag" and "Ad- pIX SnoopTagJr"

In some ~~embodiment~~examples, the combination of the second peptide partner and the attached entity for attachment/decoration, such as an antigen is of significant size, for example it over 20kDa, 25 kDa, 30 kDa, 40 kDa, 50 kDa, 60 kDa, 70 kDa, 80 kDa, 90 kDa, 110 kDa, 120 kDa, 130 kDa, 140 kDa, 150 kDa or 160 kDa, 170 kDa, 180 kDa, 190 kDa or more, such as over 200 kDa, over 300 kDa or over 400 kDa in size. Such decoration of a modified adenovirus is significant, since this type of addition is not possible using genetic fusion, due to the size limit imposed by the capsid proteins themselves. The inventors have shown that the addition of a second peptide partner entities of at least around 25-30 kDa has a beneficial effect of shielding the adenovirus from neutralising antibodies. Thus, it is preferred that the combined size of the entity attached to the second peptide partner is greater than 15kDa, preferably greater than 20kDa, optionally greater than 25 kDa in size, optionally at least 30kDa in size, in order to provide a shielding effect.

The inventors have demonstrated that with larger entities (over about 30 kDa) attached to the adenoviral particle using the methods of the present ~~invention~~disclosure, the decorated adenovirus of the present ~~invention~~ disclosure may evade the usual host immune response to the adenovirus. It is postulated that this is because the entity shields the adenovirus from antibody neutralisation by the host immune system. Evasion of host immunity to the adenoviral particle itself may be useful, since it would permit the adenovirus vector to be effective even in individuals who had previously been exposed to the parental adenovirus strain. Given that a large percentage of the human population is expected to have been previously infected by adenovirus, this would be a useful improvement over the prior art. Host immune clearance of prophylactic or therapeutic adenoviral vectors is a major obstacle to current therapeutics reaching the clinic. The present ~~invention~~ disclosure can therefore be used primarily as a way to shield any adenoviral vector from the host immune system. Advantageously, the entity attached using the technology can itself have a therapeutic effect, such as an antigen for a vaccine, or a targeting moiety for cell targeting in gene therapy or oncolytic viruses.

In other ~~embodiment~~examples, DogTag is inserted into the surface loops of the hexon capsid protein. Hexon is the major component of the adenoviral capsid proteins with approximately 720 copies per virion. This enables a display of up to 720 ligands per virion, assuming that 100% of the

hexon proteins are coupled with a peptide tag. DogTag is therefore the first peptide partner in some ~~embodiment~~examples. In one ~~embodiment~~example, DogTag is inserted into at least one HVR loop of the hexon.

In other ~~embodiment~~examples, SpyCatcher is fused to the C-terminus of adenovirus minor capsid protein pIX. The attachment of a first peptide partner for example, SpyCatcher fused to the pIX capsid protein forms the vector Ad-SpyCatcher. Upon coupling to the second peptide partner SpyTag linked to an antigen, the vaccine vector formed is called Ad-SpyCatcher:SpyTag-Antigen.

In another ~~embodiment~~example, the first peptide partner inserted into pIX may be any one of SnoopCatcher, DogCatcher, SpyTag and SnoopTagJr. The resultant adenoviruses may be termed "Ad – SnoopCatcher", "Ad-DogCatcher", "Ad-SpyTag" and "Ad-SnoopTagJr". Alternatively, these may be named "Ad pIX – SnoopCatcher", "Ad pIX -DogCatcher", "Ad pIX -SpyTag" and "Ad- pIX SnoopTagJr". Upon coupling to the respective peptide pairs, these may form: "Ad – SnoopCatcher: Snooptag", "Ad-DogCatcher: DogTag", "Ad-SpyTag:Spycatcher" and "Ad-SnoopTagJr:SnoopTag ". Any entity may be attached to the second peptide partner, such as an antigen for a vaccine composition.

As used herein the colon ":" refers to the presence of a covalent bond between the peptide pairs.

Accordingly, in another ~~aspect~~example of the present disclosure ~~invention~~ there is provided a composition comprising an adenoviral vector in accordance with the present disclosure~~invention~~. The composition is preferably immunogenic. Said adenoviral vector comprises a modified capsid protein, wherein said modification primes the vector for accepting the attachment of an entity. The modified capsid protein involves the fusion or insertion of a first peptide partner. A therapeutic or prophylactic virus may then be prepared by adding the requisite second peptide partner which is attached to an entity such as an antigen. Together the first and second peptide partners form a covalent bond either spontaneously or assisted by a third entity such as a ligase. Therefore, in the therapeutic or prophylactic composition, the capsid protein is modified by the inclusion of a first peptide partner covalently linked to a second peptide partner attached to an entity such as an antigen. If the entity attached is an antigen, the composition is a vaccine composition. This is a preferred use of the technology, since it capitalises on the highly immunogenic nature of the adenovirus itself Indeed, in Figure 10 it can be seen that attaching the antigen to the vector (in this case a peptide antigen) is advantageous when compared to the results where the peptide and adenovirus are provided together as separate entities.

In another ~~aspect~~example of the present disclosure~~invention~~ there is provided a method of producing an adenoviral vector in accordance with the present disclosure~~invention~~.

In another ~~aspect~~example there is provided a method of producing a vaccine comprising the mixing of an adenoviral vector in accordance with the present disclosure~~invention~~ with a second peptide partner attached to an antigen. Said method may require the use of a third or helper entity such as a ligase.

In a further ~~aspect~~example there is provided a method of producing an oncolytic virus comprising the mixing of an adenoviral vector in accordance with the present disclosure~~invention~~ with a second peptide partner attached to an entity such as a targeting moiety or shielding entity. Said method may require the use of a third or helper entity such as a ligase. The targeting moiety may be any suitable entity that permits the virus to be targeted to a cell or tissue type. Advantageously, said moiety also blocks the binding of host antibodies to the modified adenovirus. Alternatively, the shielding entity simply helps the already targeted oncolytic virus to escape the immune response.

In a further ~~aspect~~example there is provided a method of producing a gene therapy vector comprising the mixing of an adenoviral vector in accordance with the present disclosure~~invention~~ with a second peptide partner attached to a targeting moiety. Said method may require the use of a third or helper entity such as a ligase. The targeting moiety may be any suitable entity that permits the virus to be targeted to a cell or tissue type. Advantageously, said moiety also blocks the binding of host antibodies to the modified adenovirus. Those skilled in the art are aware of numerous gene therapy uses of adenovirus. The present ~~invention~~ disclosure may be used to shield vectors from the immune system and/or help with targeting of the vector.

In another ~~aspect~~example of the present disclosure~~invention~~ there is provided a vaccine comprising an immunogenic adenoviral vector in accordance with the present disclosure~~invention~~ for use in the prophylaxis and/or treatment of a disease. Suitably, said vaccine comprises an immunogenic adenoviral vector in accordance with any ~~aspect~~example or ~~embodiment~~example of the present disclosure~~invention~~. Suitably, the vaccine is for use in mammals, including humans and animals. Suitably the vaccine is for use in humans, for example children, adults, women of reproductive age or pregnant women. In another ~~aspect~~example, the present disclosure~~invention~~ provides a method of inducing an immunogenic response, for example a protective immune response wherein the method comprises administering a composition in accordance with any ~~aspect~~example or ~~embodiment~~example of the present disclosure~~invention~~. In a further ~~aspect~~example the present disclosure~~invention~~ provides a cancer vaccine which targets one or more

tumour-specific mutations, permitting the specific targeting of an immune response to cancer cells. Personalised cancer vaccines are also contemplated. Such vaccines include one or more antigens that are specifically present on the cancer cells for that patient. This means that the vaccine is personalised to the particular cancer the patient has. These tumour-associated or cancer-associated antigens may be neoantigens, which are antigens newly expressed by the cancer cell.

In another ~~aspect~~example of the present disclosure~~invention~~ there is provided a kit comprising an adenoviral vector in accordance with the present disclosure~~invention~~. The kit may also include the second peptide partner linked to an antigen, the second peptide partner ready for protein fusion to an antigen, or the genetic sequence for the second peptide partner to allow the genetic fusion of the second peptide partner to the gene for the antigen.

The present disclosure~~invention~~ also extends to a kit for the preparation of a vaccine, said kit comprising an adenoviral vector as described herein. Also present in said kit may be the gene sequence for the second peptide partner, ready for fusing with the antigen. A further component may be instructions for use. Also included may be a third entity necessary for facilitating the covalent bond between the peptide partner pair.

In another ~~aspect~~example of the present disclosure~~invention~~ there is provided a kit comprising an adenoviral vector in accordance with the present disclosure~~invention~~. The kit may also include the second peptide partner linked to a targeting moiety, the second peptide partner ready for protein fusion to a targeting moiety, or the genetic sequence for the second peptide partner to allow the genetic fusion of the second peptide partner to the gene for the targeting moiety.

The present ~~invention~~ disclosure also extends to a kit for the preparation of an oncolytic virus, said kit comprising an adenoviral vector as described herein. Also present in said kit may be the gene sequence for the second peptide partner, ready for fusing with the targeting moiety. A further component may be instructions for use. Also included may be a third entity necessary for facilitating the covalent bond between the peptide partner pair. The adenoviral vector in this instance would be replication competent and may be modified to include genes to ensure destruction of the infected cancer cell.

The present inventors are aware that peptide binding partners have previously been used in vaccine preparation, in particular for use in virus-like particles. However, this technology is distinct from the use of a viral vector, which may have a different utility in vaccine preparation due to the immunological response the viral vector itself generates. A viral vector is efficient at gaining entry to the cytoplasm and often the nucleus as well, which leads to an enhanced cytotoxic T cell response to viral vectors, compared to virus like particles. For many infectious diseases and for cancer, the