(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 September 2014 (18.09.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/140176 A1

(51) International Patent Classification:
C07K 16/28 (2006.01) *A61K 39/395* (2006.01)

(21) International Application Number:
PCT/EP2014/054961

(22) International Filing Date:
13 March 2014 (13.03.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/788,102 15 March 2013 (15.03.2013) US

(71) Applicant: **VACCIBODY AS** [NO/NO]; Gaustadalleen 21, NO-0349 Oslo (NO).

(72) Inventors: **BREKKE, Ole Henrik**; Torshovgt. 10A, NO-0476 Oslo (NO). **LINDEBERG, Mona Mari**; Galgeberg 3G, NO-0575 Oslo (NO). **FREDRIKSEN, Agnete Brunsvik**; Strandvn. 5, NO-2005 Rællingen (NO).

(74) Agents: **HANSEN, Carsten** et al.; Inspicos A/S, P.O. Box 45, Kogle Allé 2, DK-2970 Hørsholm (DK).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2014/140176 A1

(54) Title: TARGETING VACCINES FOR VETERINARY USE

(57) Abstract: The present invention relates to therapeutic compounds, such as vaccines against avian diseases and in particular to DNA vaccines. The invention further relates to protein construct encoding homodimeric peptides, which peptides may be released from a DNA vaccine or used separately. Further described are pharmaceutical formulations, host cells and methods for producing the vaccines, as well as methods for the treatment or prevention of various diseases in animals, such as avians, such as cancers and infectious diseases.

TARGETING VACCINES FOR VETERINARY USE

FIELD OF THE INVENTION

The present invention relates to therapeutic compounds, such as vaccines against avian diseases and in particular to DNA vaccines. The invention further relates to DNA construct encoding homodimeric peptidesas well as vector vaccines. The encoded peptides may be expressed from the DNA vaccine construct directly within the host receiving the vaccine or be produced and used as recombinant peptides separately. Further described are pharmaceutical formulations, host cells and methods for producing the vaccines, as well as methods for the treatment or prevention of various diseases in animals, such as avians, such as cancers and infectious diseases.

BACKGROUND OF THE INVENTION

The global veterinary vaccines market is expected to post substantial growth in ensuing years. Growing demand for the vaccines from Asia, Latin America and Eastern European countries and increased vulnerability of animals to the diseases is steering the demand for veterinary vaccines. Rapidly changing patterns of the diseases among the animals and increased development of resistance to the currently used antimicrobials is compelling the manufacturers to invest heavily in new product developments. Adoption of novel animal husbandry techniques and different farming conditions are attributed as the major factors for emergence of newer diseases. Growing awareness on animal health and benefits of early detection and preventive medicines will drive the demand for veterinary vaccines. Technology innovations, in particular DNA-vaccines, replicating and non-replicating vector vaccines, and introduction of new products that are capable of ensuring greater production and immune responses than traditional vaccines also offers good prospects for the future of veterinary vaccines market.

The global veterinary vaccines market ($4.23 Bn) accounted for around 20% of the total vaccines market ($29.71 Bn) in 2010. It is expected to grow with a CAGR of 5.80% to reach the market size of $5.6 Bn by 2015. United States represents the largest market for veterinary vaccines worldwide, with the market share of 46% and market size of $1.94 Bn in 2010.

The market segment for livestock vaccines accounts for approximately $2 Bn and is composed of vaccines for cattle, pigs, sheep, poultry and within aquaculture. The efforts in

vaccine development within the livestock segment are driven by the need for vaccines obtaining better, safer and more effective responses. Additionally, ways to easily administer vaccines in herds, such as for mass vaccination of chickens, both with respect to the number of doses needed to be given and methods of facile administration, is a challenging demand.

Chickens express many of the regulatory proteins that mammals do. Much effort is on-going to augment immune responses or even alter a bird's capacity to respond to vaccines. Avian dendritic cells are now being characterized and the research suggests that these cells, like their mammalian counterparts, are the key antigen presenting cell in the initiation of a robust immune response. Targeting avian dendritic cells with vaccines therefore should be an attractive approach for obtaining effective immune responses for novel chicken vaccines.

There is a need for improved vaccines against poultry diseases including avian coccidiosis, necrotic enteritis, avian encephalomyelitis, avian infectious bronchitis, avian infectious bursal disease, avian reovirus, chicken anaemia virus, duck virus enteritis, egg drop syndrome 1976, erysipelas, infectious laryngotracheitis, Marek's disease, Newcastle disease, pasteurellosis, post-natal colibacillosis, salmonellosis, swollen head syndrome, turkey haemorrhagic enteritis, turkey rhinotracheitis and avian influenza.

OBJECT OF THE INVENTION

Vaccibodies (International patent application number PCT/EP2012/076404, WO2011161244, and WO2004076489) are vaccines, DNA constructs that harbours the ability to express protein molecules targeting antigen presenting cells (APC), such as dendritic cells, *in vivo* and *in vitro* by being directed towards specific surface receptors on the APCs. Vaccibody can be delivered both as a DNA vaccine, a vector vaccine, or the encoded protein subunit vaccine. Alternatively, Vaccibody constructs within the present invention may be used to activate APC *in vitro*, and then the activated APC may be used for vaccination.

The invention describes Vaccibody vaccines based on the format described in figure 1, which are composed of a targeting protein module (herein also referred to as a targeting unit) binding to receptors expressed on the cell surface of APCs. In this specific embodiment, the targeting unit is composed of a scFv fragment that is recognising and binding chicken MHC class II molecules on avian cells. A dimerization protein module composed of hinge regions, hinge–like regions and constant heavy chain domains from human, rodent, bovine or avian immunoglobulins, which connects two vaccibody monomers generating a homodimer molecule. And the antigenic unit (vaccine module) that can be any protein unit such as a

domain, short segment or a peptide or combinations of different protein units, derived from a pathogen or cancer related tissue.

The invention describes the novel vaccine format that is designed for obtaining significant better and more effective vaccines for veterinary purposes, which can be delivered as a DNA vaccine, vector vaccine, or protein vaccines by different administration methods, such as *in ovo*, in the drinking water, as aerosol spray, delivered by jet injectors, as needle injection or as viral vector delivery methods.

It is an object of embodiments of the invention to provide specific and highly effective therapeutic compounds, such as DNA vaccines against diseases and conditions in animals, such as in avian species.

SUMMARY OF THE INVENTION

It has been found by the present inventor(s) that a scFv fragment specifically binding avian MHC class II molecule, such as chicken MHC class II molecule, on avian cells may be used as a highly efficient targeting unit in the design of vaccines in a vaccibody structure.

So, in a first aspect the present invention relates to a homodimeric protein of two identical amino acid chains, each amino acid chain comprising (1) optionally a signal peptide, (2) a targeting unit, (3) a dimerization motif, and (4) an antigenic unit, said targeting unit being a scFv fragment specifically binding avian MHC class II molecule, such as chicken MHC class II molecule, on avian cells.

It is to be understood that the constructs according to the present invention only require a signal peptide in a form where it is to be exported out of a cell producing such construct. Accordingly, a nucleic acid construct usually have to contain a sequence encoding the signal peptide to have a final protein exported from the cell producing such protein. However, if produced and administered as a recombinant protein, a signal peptide may not be required. In some embodiments, the homodimeric protein of two identical amino acid chains does not contain a signal peptide.

In a second aspect, the present invention relates to an amino acid chain comprising (1) an optional signal peptide, (2) a targeting unit, (3) a dimerization motif, and (4) an antigenic unit, said targeting unit being a scFv fragment specifically binding chicken MHC class II molecule on avian cells, which amino acid chain is able to form a homodimeric protein according to the present invention.

In a third aspect the present invention relates to a nucleic acid molecule, such as a DNA, encoding an amino acid chain comprising (1) an optional signal peptide, (2) a targeting unit, (3) a dimerization motif, and (4) an antigenic unit, said targeting unit being a scFv fragment specifically binding chicken MHC class II molecule on avian cells, which amino acid chain is able to form a homodimeric protein according to the present invention.

In a further aspect, the present invention relates to an amino acid chain comprising (1) an optional signal peptide, (2) a targeting unit, (3) an optional dimerization motif, and (4) an antigenic unit, said targeting unit being a scFv fragment specifically binding chicken MHC class II molecule on avian cells.

In a further aspect the present invention relates to a vector, such as a viral vector or a plasmid vector, such as one optimized for avians comprising the nucleic acid molecule according to the invention. In some embodiments the vector is able to express the nucleic acid molecule as a functional protein in avian cells

In a further aspect the present invention relates to a homodimeric protein according to the invention, or an amino acid chain according to the invention, or the nucleic acid molecule according to the invention or a vector according to the invention for use as a medicament.

In a further aspect the present invention relates to a pharmaceutical composition comprising a homodimeric protein according to the invention, or an amino acid chain according to the invention, or the nucleic acid molecule according to the invention, or a vector according to the invention.

In a further aspect the present invention relates to a host cell comprising the nucleic acid molecule according to the invention, or a vector according to the invention.

In a further aspect the present invention relates to a method for preparing a homodimeric protein according to the invention, or an amino acid chain of the invention, the method comprising a) transfecting the nucleic acid molecule according to the invention or a vector according to the invention into a cell population, such as eukaryotic, bacterial or yeast cells; b) culturing the cell population; c) collecting and purifying the homodimeric protein, or amino acid chain expressed from the cell population.

In a further aspect the present invention relates to a method for preparing a vaccine, such as a DNA vaccine, comprising an immunologically effective amount of a nucleic acid molecule according to the invention or a vector according to the invention, the method comprising a) preparing a nucleic acid molecule according to the invention, or a vector

according to the invention; b) dissolving the nucleic acid molecule or vector obtained under step a) in a pharmaceutically acceptable carrier, diluent, or buffer.

In a further aspect the present invention relates to a method for preparing a cell, such as an antigen presenting cell vaccine or cell line producing the homodimeric protein according to the invention, or the amino acid chain according to the invention, the method comprising; a) preparing a nucleic acid molecule according to the invention, or vector according to the invention; b) activating in vitro the cells, such as antigen presenting cells with an immunologically effective amount of a nucleic acid molecule or vector prepared under step a); and c) preparing the cells, such as antigen presenting cells obtained under step b) in a suitable diluent, such as a pharmaceutically acceptable carrier, diluent, or buffer.

In a further aspect the present invention relates to a vaccine against a disease or condition in an animal, such as a cancer or an infectious disease caused by a virus, bacteria, protozoa, or other infectious agent, the vaccine comprising an immunologically effective amount of a homodimeric protein according to the invention, or an amino acid chain according to the invention, or nucleic acid molecule, such as a DNA, according to the invention, or vector according to the invention, wherein said vaccine is able to trigger both a T-cell- and/or B-cell immune response.

In a further aspect the present invention relates to a method of treating or preventing a disease or condition in an animal, such as a cancer or an infectious disease caused by a virus, bacteria or other infectious agent, the method comprising administering to said animal in need thereof, a homodimeric protein according to the invention, or an amino acid chain according to the invention, or the nucleic acid molecule, such as a DNA, according to the invention, or vector according to the invention.

LEGENDS TO THE FIGURE

Figure 1: The vaccibody structure

Figure 2: The curve shows effective protein production and secretion of 2G11-vaccibody (absorbance at 450 nm) into the supernatant of transiently transfected HEK293E cells. OD levels observed in sandwich ELISA using anti-human IgG (MCA878) and anti-His antibodies performed on dilutions from supernatant of three independent transient transfections with 2G11 vaccibody constructs (ScFv2G11lhgD-His) and one negative control (EB=Elisa Buffer).

MCA878 recognizes the dimerization unit from human IgG3 and anti-His antibody recognized the 6x-His tag included C-terminal to the HSV-2 gD antigenic unit.

Figure 3: FFlowcytometry analysis on binding to CD45+ chicken PBMC. Isolated chicken PBMC were stained with 2G11 vaccibody and negative controls (Staining buffer, non-targeted aNIP control and isotype control). Binding analysis were performed on gated CD45+ cells using overlaying histograms.

Figure 4: Flowcytometry analysis on binding to CD45+ chicken PBMC. Isolated chicken PBMC were stained with 2G11 vaccibody and positive (2G11 mAb-AH diagnostics) and negative controls (non-targeted aNIP control and isotype control). Binding analysis were performed on gated CD45+ cells using overlaying histograms.Purple filled: 2G11 vaccibody, Green: 2G11 mAb (positive control), Pink: NlhCkCk (non-targeting vaccibody, irrelevant targeting unit), Blue line: 2G11 vaccibody isotype control

Figure 5: Flowcytometry analysis on binding to CD45+ chicken PBMC. Isolated chicken PBMC were stained with the 2G11 mAb (AH Diagnostics) and negative controls (Staining buffer and isotype control). Binding analysis were performed on gated CD45+ cells using overlaying histograms.Purple filled: 2G11 mAb (positive control), Green: Staining Buffer, Pink: 2G11 mAb isotype control.

DETAILED DISCLOSURE OF THE INVENTION

The DNA and protein constructs and DNA vaccine technology described herein by the inventors of the present invention (also referred to as "vaccibody" molecules/vaccines/constructs) represents a novel vaccine strategy to induce strong and specific immune responses for both infectious diseases, such as avian infectious diseases and cancer. The vaccine described herein may be administered as a DNA vaccine by intradermal or intramuscular or in ovo injection, or via the respiratory/mucosal/GI- tract. This results in the uptake of the DNA-construct encoding the vaccibody- vaccine in cells at the site of administration, leading to in vivo production of the vaccibody- protein molecule. In alternative aspects, the constructs according to the present invention are produced as recombinant protein vaccines and administered by similar means as the DNA vaccine.

The vaccibody molecule described herein is a homodimer consisting of three modules; targeting module (or unit), dimerization module (or motif) and the antigenic unit (vaccine module) (Figure 1). Genes encoding the three modules are genetically engineered to be expressed as one gene translated to one protein. When expressed in vivo, the vaccibody

molecule targets MHC class II molecules on antigen-presenting cells which may result in enhanced vaccine potency compared to identical, non-targeted antigens.

The invention describes several variants of Vaccibody vaccines, all based on the overall format described in figure 1. The vaccibody vaccines is composed of a *targeting unit* encoding a scFv fragment specifically binding chicken MHC class II molecule on avian cells. The *dimerization module* genes may encode hinge regions and constant heavy chains, such as domains from avian IgY which connects two vaccibody monomers generating a homodimer molecule. Genes encoding the antigenic unit (vaccine module) for the current strategy may be for any protein unit such as a domain, short segment or a peptide or combinations of different protein units, derived from a pathogen or cancer related tissue. Once the DNA vaccine is administered *in vivo,* cells receiving the vaccine construct will express the vaccibody proteinmolecule. The *in vivo* produced vaccibody vaccines target MHC class II molecule expressed on the surface of APCs. The binding of the vaccibody molecule to its cognate receptors leads to internalization of the complex in the APC, degradation of the proteins into small peptides that are loaded onto MHC molecules and presented to $CD4^+$ and $CD8^+$ T cells to induce a specific immune response to the antigenic unit. Once stimulated and with help from activated $CD4^+$ T cells, $CD8^+$ T cells will target and kill antigen expressing cells. Such enhanced immune responses to a vaccine with a "built-in" adjuvant effect may potentially overcome tumor-escape by breaking immunological tolerance and efficiently kill malignant cells, or enhancing the immune response to pathogens. The targeting unit by the scFv fragment specifically binding chicken MHC class II molecule may be connected through a dimerization motif, such as a hinge region/shortened CH2 domain, to an antigenic unit, wherein the later is in either the COOH-terminal or the NH2-terminal end. The present invention not only relates to a DNA sequence coding for this recombinant protein, but also to expression vectors comprising these DNA sequences, cell lines comprising said expression vectors, to treatment of mammals and avians preferentially by immunization by means of Vaccibody DNA, Vaccibody RNA, or Vaccibody protein, and finally to pharmaceuticals and a kit comprising the said molecules.

As used herein the terms "treatment" or "treating" refers to preventing, alleviating, managing, curing or reducing one or more symptoms or clinically relevant manifestations of a disease or disorder, unless contradicted by context. For example, "treatment" of a subject, such as an avian population in whom no symptoms or clinically relevant manifestations of a disease or disorder have been identified is preventive or prophylactic therapy, whereas "treatment" of subjects in which symptoms or clinically relevant manifestations of a disease or disorder have been identified generally does not constitute preventive or prophylactic therapy.The dimerization motif in the proteins according to the present invention may be constructed to include a hinge region and/or immunoglobulin domains (e.g. Cγ3 domain from human IgG3 or

C_H2 and/or C_H4 domain of avian IgY, or a sequence that is substantially identical to said C domain. The hinge region may be Ig, such as IgG derived and contributes to the dimerization through the formation of an interchain covalent bond(s), e.g. disulfide bridge(s). In addition, it functions as a flexible spacer between the domains allowing the two targeting units to bind simultaneously to two target receptors on APC expressed with variable distances. The immunoglobulin domains contribute to homodimerization through non-covalent interactions, e.g. hydrophobic interactions. In one embodiment the dimerization motif comprises a C_H3 domain, such as one derived from IgG. In one embodiment the dimerization motif comprises a C_H4 domain, such as one derived from IgY. These dimerization motifs may be exchanged with other multimerization moieties (e.g. from other Ig isotypes/subclasses). Preferably the dimerization motif is derived from native proteins, such as IgG, IgM or IgY molecules from relevant species, such as from an avian species, such as from chicken.

It is to be understood that the dimerization motif may have any orientation with respect to antigenic unit and targeting unit. In one embodiment the antigenic unit is in the COOH-terminal end of the dimerization motif with the targeting unit in the N-terminal end of the dimerization motif. In another embodiment the antigenic unit is in the N-terminal end of the dimerization motif with the targeting unit in the COOH-terminal end of the dimerization motif.

International application WO 2004/076489, PCT/EP2012/076404, and WO2011161244, which is hereby incorporated by reference discloses nucleic acid sequences and vectors, which may be used according to the present invention.

The proteins according to the present invention include an antigenic unit, as well as immunogenic fragments or variants thereof. The antigenic sequence should be of sufficient length. The minimal length of such antigenic unit may be around 9 amino acids. Accordingly in some embodiments, the antigenic unit comprises an amino acid sequence of at least 9 amino acids corresponding to at least about 27 nucleotides in a nucleic acids sequence encoding such antigenic unit.

Immunization by means of Vaccibody protein, Vaccibody DNA, or Vaccibody RNA, the latter two executed e.g. by intramuscular or intradermal injection with or without a following electroporation, are all feasible methods according to the present invention.

As discussed above, the present invention relates to a vaccine composition that may be used against any cancer or infectious diseases, the vaccine composition comprising an immunologically effective amount of the nucleic acid encoding the molecule of the invention or degenerate variants thereof. The vaccine may be able to trigger both a T-cell- and B-cell

immune response. The present invention also relates to a kit comprising Vaccibody DNA, RNA, or protein for diagnostic, medical or scientific purposes.

The invention further relates to a method of preparing the recombinant molecule of the invention comprising, transfecting the vector comprising the molecule of the invention into a cell population; culturing the cell population; collecting recombinant protein expressed from the cell population; and purifying the expressed protein.

The above described nucleotide sequences may be inserted into a vector suited for gene therapy, e.g. under the control of a specific promoter, and introduced into the cells. It is to be understood that the term vector as used herein refers to any molecule or construct suitable for delivering the nucleotide sequences according to the present invention. In some embodiments the vector is a plasmid vector. In some embodiments the vector is a viral vector.

In some embodiments the vector comprising said nucleotide sequence is a virus (a viral vector), e.g. Lentivirus, an adenovirus, alphavirus, herpes, vaccinia virus or an adeno-associated virus, or alternatively avian vectors derived from HVT, fowl pox, newcastle or any other avian vector. In some embodiments a retroviruse is used as vector. Examples of suitable retroviruses are e.g. MoMuLV or HaMuSV. For the purpose of gene therapy, the DNA/RNA sequences according to the invention can also be transported to the target cells in the form of colloidal dispersions. They comprise e.g. liposomes or lipoplexes. Nonviral substances such as Ormosil may also be used as vectors and may deliver nucleotide sequences to specifically targeted cells in living animals.

The present invention encompasses the use of a targeting unit, an antigenic unit, as well as a dimerization motif comprising a hinge region and optionally carboxyterminal C domains and linkers, each domain having minimum degree of sequence identity or sequence homology with amino acid sequence(s) defined herein or with a polypeptide having the specific properties defined herein. The present invention encompasses, in particular, the use of peptide variants or peptide units to be used in the constructs according to the present invention having a degree of sequence identity with any one of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, or SEQ ID NO:40. Here, the term "variant" means an entity having a certain degree of sequence identity with the subject amino acid sequences or the subject nucleotide sequences, where the subject amino acid sequence preferably is SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16,

SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, or SEQ ID NO:40.

In one aspect, the variant or fragment amino acid sequence and/or nucleotide sequence should provide and/or encode a polypeptide which retains the functional activity and/or enhances the activity of a polypeptide of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, or SEQ ID NO:40.

In the present context, a variant sequence is taken to include an amino acid sequence which may be at least 70%, 75%, 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99%, identical to the subject sequence. Typically, the variants used according to the present invention will comprise the same active sites etc. as the subject amino acid sequence. Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of the present invention it is preferred to express homology in terms of sequence identity.

Sequence identity comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison computer programs. These commercially available computer programs use complex comparison algorithms to align two or more sequences that best reflect the evolutionary events that might have led to the difference(s) between the two or more sequences. Therefore, these algorithms operate with a scoring system rewarding alignment of identical or similar amino acids and penalising the insertion of gaps, gap extensions and alignment of non-similar amino acids. The scoring system of the comparison algorithms include:

i) assignment of a penalty score each time a gap is inserted (gap penalty score),

ii) assignment of a penalty score each time an existing gap is extended with an extra position (extension penalty score),

iii) assignment of high scores upon alignment of identical amino acids, and

iv) assignment of variable scores upon alignment of non-identical amino acids.

Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons.

The scores given for alignment of non-identical amino acids are assigned according to a scoring matrix also called a substitution matrix. The scores provided in such substitution matrices are reflecting the fact that the likelihood of one amino acid being substituted with another during evolution varies and depends on the physical/chemical nature of the amino acid to be substituted. For example, the likelihood of a polar amino acid being substituted with another polar amino acid is higher compared to being substituted with a hydrophobic amino acid. Therefore, the scoring matrix will assign the highest score for identical amino acids, lower score for non-identical but similar amino acids and even lower score for non-identical non-similar amino acids. The most frequently used scoring matrices are the PAM matrices (Dayhoff et al. (1978), Jones et al. (1992)), the BLOSUM matrices (Henikoff and Henikoff (1992)) and the Gonnet matrix (Gonnet et al. (1992)).

Suitable computer programs for carrying out such an alignment include, but are not limited to, Vector NTI (Invitrogen Corp.) and the ClustalV, ClustalW and ClustalW2 programs (Higgins DG & Sharp PM (1988), Higgins et al. (1992), Thompson et al. (1994), Larkin et al. (2007). A selection of different alignment tools is available from the ExPASy Proteomics server at www.expasy.org. Another example of software that can perform sequence alignment is BLAST (Basic Local Alignment Search Tool), which is available from the webpage of National Center for Biotechnology Information which can currently be found at http://www.ncbi.nlm.nih.gov/ and which was firstly described in Altschul et al. (1990) J. Mol. Biol. 215; 403-410.

Once the software has produced an alignment, it is possible to calculate % similarity and % sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

In one embodiment, it is preferred to use the ClustalW software for performing sequence alignments. Preferably, alignment with ClustalW is performed with the following parameters for pairwise alignment:

Substitution matrix:	Gonnet 250
Gap open penalty:	20
Gap extension penalty:	0.2
Gap end penalty:	None

ClustalW2 is for example made available on the internet by the European Bioinformatics Institute at the EMBL-EBI webpage www.ebi.ac.uk under tools – sequence analysis –

ClustalW2. Currently, the exact address of the ClustalW2 tool is www.ebi.ac.uk/Tools/clustalw2.

In another embodiment, it is preferred to use the program Align X in Vector NTI (Invitrogen) for performing sequence alignments. In one embodiment, Exp10 may be used with default settings:
Gap opening penalty: 10
Gap extension penalty: 0.05
Gap separation penalty range: 8
Score matrix: blosum62mt2

Thus, the present invention also encompasses the use of variants, fragments, and derivatives of any amino acid sequence of a protein, polypeptide, motif or domain as defined herein, particularly those of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, or SEQ ID NO:40.

The sequences, particularly those of variants, fragments, and derivatives of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, or SEQ ID NO:40, may also have deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent substance. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the secondary binding activity of the substance is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine, valine, glycine, alanine, asparagine, glutamine, serine, threonine, phenylalanine, and tyrosine.

The present invention also encompasses conservative substitution (substitution and replacement are both used herein to mean the interchange of an existing amino acid residue, with an alternative residue) that may occur i.e. like-for-like substitution such as basic for basic, acidic for acidic, polar for polar etc. Non-conservative substitution may also occur i.e. from one class of residue to another or alternatively involving the inclusion of unnatural

amino acids such as ornithine (hereinafter referred to as Z), diaminobutyric acid ornithine (hereinafter referred to as B), norleucine ornithine (hereinafter referred to as O), pyriylalanine, thienylalanine, naphthylalanine and phenylglycine.

Conservative substitutions that may be made are, for example within the groups of basic amino acids (Arginine, Lysine and Histidine), acidic amino acids (glutamic acid and aspartic acid), aliphatic amino acids (Alanine, Valine, Leucine, Isoleucine), polar amino acids (Glutamine, Asparagine, Serine, Threonine), aromatic amino acids (Phenylalanine, Tryptophan and Tyrosine), hydroxyl amino acids (Serine, Threonine), large amino acids (Phenylalanine and Tryptophan) and small amino acids (Glycine, Alanine).

Replacements may also be made by unnatural amino acids include; alpha* and alpha-disubstituted* amino acids, N-alkyl amino acids*, lactic acid*, halide derivatives of natural amino acids such as trifluorotyrosine*, p-Cl-phenylalanine*, p-Br-phenylalanine*, p-I-phenylalanine*, L-allyl-glycine*, ß-alanine*, L-α-amino butyric acid*, L-γ-amino butyric acid*, L-α-amino isobutyric acid*, L-ε-amino caproic acid$^{\#}$, 7-amino heptanoic acid*, L-methionine sulfone$^{\#*}$, L-norleucine*, L-norvaline*, p-nitro-L-phenylalanine*, L-hydroxyproline$^{\#}$, L-thioproline*, methyl derivatives of phenylalanine (Phe) such as 4-methyl-Phe*, pentamethyl-Phe*, L-Phe (4-amino)$^{\#}$, L-Tyr (methyl)*, L-Phe (4-isopropyl)*, L-Tic (1,2,3,4-tetrahydroisoquinoline-3-carboxyl acid)*, L-diaminopropionic acid$^{\#}$ and L-Phe (4-benzyl)*. The notation * has been utilised for the purpose of the discussion above (relating to homologous or non-conservative substitution), to indicate the hydrophobic nature of the derivative whereas # has been utilised to indicate the hydrophilic nature of the derivative, #* indicates amphipathic characteristics.

Variant amino acid sequences may include suitable spacer groups that may be inserted between any two amino acid residues of the sequence including alkyl groups such as methyl, ethyl or propyl groups in addition to amino acid spacers such as glycine or β-alanine residues. A further form of variation, involves the presence of one or more amino acid residues in peptoid form, will be well understood by those skilled in the art. For the avoidance of doubt, "the peptoid form" is used to refer to variant amino acid residues wherein the α-carbon substituent group is on the residue's nitrogen atom rather than the α-carbon. Processes for preparing peptides in the peptoid form are known in the art, for example Simon RJ *et al.* (1992), Horwell DC. (1995).

In one embodiment, the variant targeting unit used in the homodimeric protein according to the present invention is variant having the sequence of amino acids at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% amino acid sequence identity therewith.

In one aspect, preferably the protein or sequence used in the present invention is in a purified form. The term "purified" means that a given component is present at a high level without significant levels of other protein or sequences, such as being 99%, 95%, 90%, 85%, 80%, 75%, or 70% pure. The component is desirably the predominant active component present in a composition.

A "variant" or "variants" refers to proteins, polypeptides, units, motifs, domains or nucleic acids. The term "variant" may be used interchangeably with the term "mutant." Variants include insertions, substitutions, transversions, truncations, and/or inversions at one or more locations in the amino acid or nucleotide sequence, respectively. The phrases "variant polypeptide", "polypeptide", "variant" and "variant enzyme" mean a polypeptide/protein that has an amino acid sequence that has been modified from the amino acid sequence of SEQ ID NO: 1. The variant polypeptides include a polypeptide having a certain percent, e.g., 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, of sequence identity with the amino acid sequence of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, or SEQ ID NO:40.

"Variant nucleic acids" can include sequences that are complementary to sequences that are capable of hybridizing to the nucleotide sequences presented herein. For example, a variant sequence is complementary to sequences capable of hybridizing under stringent conditions, e.g., 50°C and 0.2X SSC (1X SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), to the nucleotide sequences presented herein. More particularly, the term variant encompasses sequences that are complementary to sequences that are capable of hybridizing under highly stringent conditions, e.g., 65°C and 0.1X SSC, to the nucleotide sequences presented herein. The melting point (Tm) of a variant nucleic acid may be about 1, 2, or 3ºC lower than the Tm of the wild-type nucleic acid. The variant nucleic acids include a polynucleotide having a certain percent, e.g., 80%, 85%, 90%, 95%, or 99%, of sequence identity with the nucleic acid encoding SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, or SEQ ID NO:40, encoding the monomeric protein which can form the homodimeric protein according to invention.

Signal peptide:

A signal peptide at the N-terminal end of the nascent polypeptide directs the molecule into the ER before transport into the Golgi complex. The signal peptide is cleaved off by signal peptidase once it has served its purpose of targeting and importing the protein to the ER. These signal peptides are generally between 15 and 30 amino acids, but can have more than 50 residues *(Martoglio, B. et al., Trends in Cell Biology, 1998, Knappskog, S. et al., J Biotechnol, 2007)*. The native signal peptide may be replaced by signal peptides from any mammalian, avian, prokaryotic or marine origin. Commonly used signal peptides are e.g. humanIL-2 and human albumin due to their natural ability to secrete large amounts of protein. The choice of signal peptide can have a considerable impact on the amount of synthesized and secreted protein.

In some embodiments the signal peptide is not derived from pLNOH2 (B1-8 variable immunoglobulin leader) disclosed in the international application with International Application No: PCT/EP2011/060628.

In some embodiments the signal peptide is not derived from an immunoglobulin gene.

In some embodiments the signal peptide is derived from classes of peptides known to effectively secrete proteins from avian cells, such as chicken IL-2 signal peptide or chicken immunoglobulin signal peptides.

Homodimeric protein:

The term "homodimeric protein" as used herein refers to a protein comprising two individual identical strands of amino acids, or subunits held together as a single, dimeric protein by hydrogen bonding, ionic (charged) interactions, actual covalent disulfide bonding, or some combination of these interactions.

The term "dimerization motif", as used herein, refers to the sequence of amino acids between the antigenic unit and the targeting unit comprising the hinge region/ shortened CH2 domain and the optional second domain that may contribute to the dimerization. This dimerisation motif may be of immunoglobulin origin, and optionally the hinge region/ shortened CH2 domain and the second domain are connected through a linker. Accordingly the dimerization motif serves to connect the antigenic unit and the targeting unit, but also facilitates the dimerization of the two monomeric proteins into a homodimeric protein according to the invention.

It is to be understood that for some aspects of the present invention, wherein the construct only contain a single amino acid chain comprising optionally a signal peptide, a targeting unit, and an antigenic unit, then a dimerization motif may be absent from the construct.

Targeting unit:

The term "targeting unit" as used herein refers to a unit that delivers the protein with its antigen to mammalian or avian APC for MHC class II-restricted presentation to CD4+ T cells or for providing cross presentation to CD8+ T cells by MHC class I restriction. The targeting unit used in the constructs according to the present invention is a single chain Fv fragment specifically binding chicken MHC class II molecules on avian cells, such as one derived from the hybridoma MaD2G11.

Antigenic unit:

The term "antigenic unit" as used herein refers to any molecule, such as a peptide which is able to be specifically recognized by an antibody or other component of the immune system, such as a surface receptor on T-cells. Included within this definition are also immunogens that are able to induce an immune response. The terms "epitope" or "antigenic epitope" is used to refer to a distinct molecular surface, such as a molecular surface provided by a short peptide sequence within an antigenic unit. In some embodiments the antigenic unit comprises two or more antigenic epitopes. The antigenic unit used in the constructs according to the present invention may be derived from herpes simplex virus 2 glycoprotein D (gD), but any antigenic unit suitable for preventive and/or therapeutic effect for e.g. poultry diseases may be utilized This includes antigens from agents causing avian coccidiosis, avian encephalomyelitis, avian infectious bronchitis, such as S protein, S1 protein or S2 protein, avian infectious bursal disease, such as VP2 protein, avian reovirus, such as Sigma Cc protein, chicken anaemia virus, duck virus enteritis, egg drop syndrome 1976, erysipelas, infectious laryngotracheitis, Marek's disease, Newcastle disease, such as Hemagglutinin-neuraminidase fusion protein pasteurellosis, post-natal colibacillosis, salmonellosis, swollen head syndrome, turkey haemorrhagic enteritis, turkey rhinotracheitis and avian influenza, such as HA proteins, such as HA1, HA2, HA3, HA4, HA5, HA6, HA7, HA8, HA9, HA10, HA11, HA12, HA13, HA14, or HA15.

The term "hinge region" refers to a peptide sequence of the homodimeric protein that facilitates the dimerization, such as through the formation of an interchain covalent bond(s), e.g. disulfide bridge(s). The hinge region may be Ig derived, such as hinge exons h1+h4 of an Ig, such as IgG3, or equivalent units derived from avian immunoglobulin molecules, e.g. CH2 domain from IgY.

Formulations

The DNA constructs and encoded proteins of the invention can be formulated using one or more excipients to increase stability; increase cell transfection; permit a sustained or delayed release; increase the translation of encoded protein in vivo; and/or alter the release profile of encoded protein in vivo. In addition to traditional excipients such as any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, excipients of the present invention can include, without limitation, lipidoids, liposomes, lipid nanoparticles, polymers, lipoplexes, core-shell nanoparticles, peptides, proteins, cells transfected with modified nucleic acid, hyaluronidase, nanoparticle mimics and combinations thereof.

Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of associating the active ingredient with an excipient and/or one or more other accessory ingredients.

A pharmaceutical composition in accordance with the present disclosure may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a "unit dose" refers to a discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient may generally be equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage including, but not limited to one-half or one-third of such a dosage.

Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the present invention may vary, depending upon the identity, size, and/or condition of the population being treated and further depending upon the route by which the composition is to be administered. For example, the composition may comprise between 0.1% and 99% (w/w) of the active ingredient.

Pharmaceutical formulations may additionally comprise a pharmaceutically acceptable excipient, which, as used herein, includes, but is not limited to, any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, and the like, as suited to the particular dosage form desired. Various excipients for formulating pharmaceutical compositions and techniques for preparing the composition are known in the art (see Remington: The Science and Practice of Pharmacy, 21.sup.st Edition, A. R. Gennaro,

Lippincott, Williams & Wilkins, Baltimore, Md., 2006; incorporated herein by reference in its entirety).

Specific embodiments of the invention

A stated above the present invention relates to a homodimeric protein of two identical amino acid chains, each amino acid chain comprising (1) optionally a signal peptide, (2) a targeting unit, (3) a dimerization motif, and (4) an antigenic unit, said targeting unit being a scFv fragment specifically binding avian MHC class II molecule, such as chicken MHC class II molecule, on avian cells.

In some embodiments, the targeting unit comprises an amino acid sequence having at least 80 % sequence identity to the amino acid sequence of SEQ ID NO:7.

In some embodiments, the targeting unit comprises an amino acid sequence having at least 80 % sequence identity to the amino acid sequence of SEQ ID NO:9.

In some embodiments, the targeting unit comprises an amino acid sequence having at least 80 % sequence identity to the amino acid sequence of SEQ ID NO:11.

In some embodiments, the antigenic unit is any protein unit such as a domain, short segment or a peptide or combinations of different protein units, derived from a pathogen or cancer related tissue.

In some embodiments, the targeting unit, dimerization motif and antigenic unit in said amino acid chain are in the N-terminal to C-terminal order of targeting unit, dimerization motif and antigenic unit.

In some embodiments, the targeting unit comprises an amino acid sequence having at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity to the amino acid sequence of SEQ ID NO:7.

In some embodiments, the targeting unit comprises an amino acid sequence having at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least

93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity to the amino acid sequence of SEQ ID NO:9.

In some embodiments, the targeting unit consists of an amino acid sequence having at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity to the amino acid sequence of SEQ ID NO:11.

In some embodiments, the dimerization motif comprises a hinge region and optionally another domain that facilitate dimerization, such as an immunoglobulin domain, optionally connected through a linker.

In some embodiments, the hinge region is Ig derived, such as a shortened CH2 domain from IgY, such as an Ig derived from avian, such as chicken.

In some embodiments, the hinge region/shortened CH2 domain has the ability to form one, two, or several covalent bonds.

In some embodiments, the covalent bond is a disulphide bridge.

In some embodiments, the immunoglobulin domain of the dimerization motif is a carboxyterminal C domain, such as a constant CH3 or CH4 domain, or a sequence that is substantially identical to said C domain or a variant or a functional fragment thereof.

In some embodiments, the carboxyterminal C domain is derived from IgY or IgG.

In some embodiments, the immunoglobulin domain of the dimerization motif has the ability to homodimerize.

In some embodiments, the immunoglobulin domain has the ability to homodimerize via noncovalent interactions. In some embodiments, the noncovalent interactions are hydrophobic interactions.

In some embodiments, the dimerization domain comprise two domains selected from CH2, and CH4 or functional fragment thereof, such as a CH2 and/or a CH4 domain or functional fragment thereof from IgY.

In some embodiments, the dimerization motif consist of hinge exons h1 and h4 connected through a linker to a C_H3 domain of human or mouse, IgG, such as IgG3 or shortened version of CH2 connected to C_H4 domain of avian IgY.

In some embodiments, the dimerization motif consist of an amino acid sequence having at least 80 % sequence identity to the amino acid sequence of SEQ ID NO:13.

In some embodiments, the linker is a $G_3S_2G_3SG$ linker.

In some embodiments, the antigenic unit and the dimerization motif is connected through a linker, such as a GLGGL linker or a GLSGL linker.

In some embodiments, the targeting unit consists of SEQ ID NO:11, or a variant thereof.

In some embodiments, the homodimeric protein binds specifically to chicken MHC class II molecule on avian cells with a K_d lower than 0,1 nM, such as lower than 50 pM, such as lower than 40, 30, 20 or 10 pM.

In some embodiments, the antigenic unit comprises an antigenic unit suitable for preventive and/or therapeutic effect for poultry diseases, such as an antigen selected from agents causing avian coccidiosis, avian encephalomyelitis, avian infectious bronchitis, such as S protein, S1 protein or S2 protein, avian infectious bursal disease, such as VP2 protein, avian reovirus, such as Sigma Cc protein, chicken anaemia virus, duck virus enteritis, egg drop syndrome 1976, erysipelas, infectious laryngotracheitis, Marek's disease, Newcastle disease, such as Hemagglutinin-neuraminidase fusion proteinhemagglutinin-neuraminidase fusion protein, pasteurellosis, post-natal colibacillosis, salmonellosis, swollen head syndrome, turkey haemorrhagic enteritis, turkey rhinotracheitis and avian influenza, such as HA proteins, such as HA1, HA2, HA3, HA4, HA5, HA6, HA7, HA8, HA9, HA10, HA11, HA12, HA13, HA14, or HA15.

In some embodiments, the antigenic unit comprises an amino acid sequence having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least

92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity to any one amino acid sequence selected from SEQ ID NO:23 SEQ ID NO:30, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, or SEQ ID NO:40, or a fragment thereof.

In some embodiments, the antigenic unit consists of an amino acid sequence having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity to any one amino acid sequence selected from SEQ ID NO:23 SEQ ID NO:30, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, or SEQ ID NO:40, or a fragment thereof.

As used herein a fragment refers to a functional subsequence of a given sequence, usually with more than 10 amino acids, such as with more than 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 280, 300, 320, 340, 360, 380, 400, 420, 440, 460, 480, 500, 520, or 540 amino acids. For fragment used as antigenic units this fragment needs to be antigenic.

In some embodiments, the homodimeric protein according to the present invention is in its mature form without any signal peptide sequence.

In some embodiments, the avian are poultry, such as any one selected from Chicken, Duck, Emu, Goose, Indian Peafowl, Mute Swan, Ostrich, Pigeon, Turkey, Guineafowl, Common Pheasant, Golden Pheasant, and Rhea. In some embodiments, the avian is Chicken.

In some embodiments, the homodimeric protein according to the present invention ccomprises an amino acid sequence having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity to any one amino acid sequence selected from the hCH3 IgG3 domain of SEQ ID NO:17, the hinge region of SEQ ID NO:14, the hinge region of SEQ ID NO:15, the linker of SEQ ID NO:16, SEQ ID NO:18, or SEQ ID NO:19, the hinge region of SEQ ID NO:20 or SEQ ID NO:21, the CH3 hIgG3 domain of SEQ ID NO:22, the construct of SEQ ID NO:24, SEQ ID NO:31, or SEQ ID NO:32, the Chicken IgY CH2

domain of SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, or SEQ ID NO:29, the Chicken IgY CH4 of SEQ ID NO:26, or any functional fragment thereof.

Another aspect of the present invention relates to an amino acid chain comprising (1) an optional signal peptide, (2) a targeting unit, (3) a dimerization motif, and (4) an antigenic unit, said targeting unit being a scFv fragment specifically binding chicken MHC class II molecule on avian cells, which amino acid chain is able to form a homodimeric protein according to the invention. Another aspect of the present invention relates to a nucleic acid molecule, such as a DNA, encoding such amino acid chain. In some embodiments, the nucleic acid molecule is avian codon optimized.

Another aspect of the present invention relates to a nucleic acid molecule comprising or consisting of any one of nucleotide sequences selected from the list consisting of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, nucleotides 1-36 of SEQ ID NO:12, nucleotides 37-81 of SEQ ID NO:12, nucleotides 82-111 of SEQ ID NO:12, nucleotides 112-432 of SEQ ID NO:12, nucleotides 433-447 of SEQ ID NO:12, or a variant thereof.

In some embodiments, the nucleic acid molecule according to the invention is comprised by a vector. In some embodiments, the nucleic acid molecule according to the invention is formulated for administration to a subject, such as an avian population to induce production of the homodimeric protein in said subject, such as an avian population.

Another aspect of the present invention relates to a homodimeric protein according to the invention, or an amino acid chain according to the invention, or the nucleic acid molecule according to the invention for use as a medicament.

Another aspect of the present invention relates to a pharmaceutical composition comprising the homodimeric protein according to the invention, or an amino acid chain according to the invention, or the nucleic acid molecule according to the invention.

Another aspect of the present invention relates to a host cell comprising the nucleic acid molecule according to the invention.

Another aspect of the present invention relates to a method for preparing a homodimeric protein according to the invention, or an amino acid chain according to the invention, the method comprising

transfecting the nucleic acid molecule according to the invention into a cell population;

culturing the cell population;

collecting and purifying the homodimeric protein, or amino acid chain expressed from the cell population.

A method for preparing a vaccine, such as a DNA vaccine, comprising an immunologically effective amount of a nucleic acid molecule according to the invention, the method comprising

preparing a nucleic acid molecule according to the invention;

dissolving the nucleic acid molecule obtained under step a) in a pharmaceutically acceptable carrier, diluent, or buffer.

Another aspect of the present invention relates to a vaccine against a disease or condition in an animal, such as a cancer or an infectious disease caused by a virus, bacteria or other infectious agent, the vaccine comprising an immunologically effective amount of a homodimeric protein according to the invention, or an amino acid chain according to the invention, or nucleic acid molecule, such as a DNA, according to the invention, wherein said vaccine is able to trigger both a T-cell- and B-cell immune response.

In some embodiments, the vaccine according to the invention further comprising a pharmaceutically acceptable carrier and/or adjuvant.

Another aspect of the present invention relates to a method of treating or preventing a disease or condition in an animal, such as a cancer or an infectious disease caused by a virus, bacteria or other infectious agent, the method comprising administering to said animal in need thereof, a homodimeric protein according to the invention, or an amino acid chain according to the invention, or the nucleic acid molecule, such as a DNA, according to the invention. In some embodiments, the method comprises administering to the subject, such as an avian population in need thereof of a nucleic acid molecule, such as a DNA, according to the invention with a subsequent step of electroporation. In some embodiments, the administration is performed intra-dermal or intra-muscular, respiratorial, mucosal, via the GI tract, or in ovo.

Sequences:

Oligonucleotide sequences:

IgκcDNAprimer: (atcaggacagcaaagacagca) TGCTGTCTTTGCTGTCCTGAT (SEQ ID NO:1)

3´IgκC rev: (CAAGAAGCACACGACTGAGGC) gcctcagtcgtgtgcttcttg (SEQ ID NO:4)

PolyG_NotI_frwd: ATATGCGGCCGCGGGGGGGGGGGGGGG (SEQ ID NO:2)

3´-mIgG1rev: ttg acc agg cat ccc agg gtc (SEQ ID NO:3)

VHL1: ggt gtg cat tcc atg gac tgg acc tgg agg (SEQ ID NO:5)

SEQ ID NO:6: 2G11 VH nucleotide sequence:
gaggtgaagctggtggagtcaggacctagcctcgtgaaaccttctcagactctgtccctc
acctgttctgtcacgggcgactccatcaccagtggttattggaactggatccggaaattc
ccagggaaaaaacttgaatacatggggttcataagctacagtggtgacacttattacaat
ccatctctcaaaagtcgaatctccatcactcgagacacatccaagaaccagtaccacctg
cagttgaattctgtgacttctgaggacacagcaacatattactgtgcaagaaggaactac
gttagtaactacgggggtggtacttcggtgtctggggcgcagggaccacggtcaccgtc
tcctca

SEQ ID NO:7: 2G11 VH amino acid sequence:
EVKLVESGPSLVKPSQTLSLTCSVTGDSITSGYWNWIRKFPGKKLEYMGFISYSGDTYYN
PSLKSRISITRDTSKNQYHLQLNSVTSEDTATYYCARRNYVSNYGGWYFGVWGAGTTVTV
SS

SEQ ID NO:8: 2G11 VL nucleotide sequence:
aacattgtaatgacccaatctcccaaatccatgtccatgtcagtaggagagagagtcacc
ctgagctgcaaggccagtgagaatgtggttacttatgtatcctggtatcaacagaaacca
gatcagtctcctaaactgctgatatacggggcatccaaccggtacactggggtccctgat
cgcttcacaggcagtggatctgcaacagatttcactcttatcatcagcagtgttcaggct
gaagaccttgcagattatcactgtggacagagttacacctatcctcccacgttcggtgct
gggaccaagctggagctgaaa

SEQ ID NO:9: 2G11 VL amino acid sequence:
NIVMTQSPKSMSMSVGERVTLSCKASENVVTYVSWYQQKPDQSPKLLIYGASNRYTGVPD
RFTGSGSATDFTLIISSVQAEDLADYHCGQSYTYPPTFGAGTKLELK

SEQ ID NO:10:2G11 ScFv nucleotide sequence:

aacattgtaatgacccaatctcccaaatccatgtccatgtcagtagga
gagagagtcaccctgagctgcaaggccagtgagaatgtggttacttatgtatcctggtat
caacagaaaccagatcagtctcctaaactgctgatatacggggcatccaaccggtacact
ggggtccctgatcgcttcacaggcagtggatctgcaacagatttcactcttatcatcagc
agtgttcaggctgaagaccttgcagattatcactgtggacagagttacacctatcctccc
acgttcggtgctgggaccaagctggagctgaaaggtggaggcggatctggcggaggtggc
tctggcggtggcggatcggaggtgaagctggtggagtcaggacctagcctcgtgaaacct

tctcagactctgtccctcacctgttctgtcacgggcgactccatcaccagtggttattgg
aactggatccggaaattcccagggaaaaaacttgaatacatggggttcataagctacagt
ggtgacacttattacaatccatctctcaaaagtcgaatctccatcactcgagacacatcc
aagaaccagtaccacctgcagttgaattctgtgacttctgaggacacagcaacatattac
tgtgcaagaaggaactacgttagtaactacgggggtggtacttcggtgtctggggcgca
gggaccacggtcaccgtctcctca

SEQ ID NO:11: the 2G11 ScFv coding aminoacid sequence (SEQ ID NO:9 followed by linker in bold and then SEQ ID NO:7):
NIVMTQSPKSMSMSVGERVTLSCKASENVVTYVSWYQQKPDQSPKLLIYGASNRYTGVPDRFTGSGSAT
DFTLIISSVQAEDLADYHCGQSYTYPPTFGAGTKLELK**GGGGSGGGGSGGGGS**EVKLVESGPSLVKPSQ
TLSLTCSVTGDSITSGYWNWIRKFPGKKLEYMGFISYSGDTYYNPSLKSRISITRDTSKNQYHLQLNSVTS
EDTATYYCARRNYVSNYGGWYFGVWGAGTTVTVSS

SEQ ID NO:12 (Hinge h1+hinge h4, Gly-Ser linker and Gly-Leu linker in bold and hCH3 IgG3 domains suitable for constructs according to the present invention)
GAGCTCAAAACCCCACTTGGTGACACAACTCACACAGAGCCCAAATCTTGTGACACACCTCCCCCGTGCCCAAGGTGCC
CA**GGCGGTGGAAGCAGCGGAGGTGGAAGTGGA**GGACAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGA
GGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAG
AGCAGCGGGCAGCCGGAGAACAACTACAACACCACGCCTCCCATGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCA
AGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACATCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCG
CTTCACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA***GGCCTCGGTGGCC*****TG**

SEQ ID NO:13 (Hinge h1 (SEQ ID NO:14), Hinge h4 (SEQ ID NO:15), linker in bold and hCH3 IgG3 domains (SEQ ID NO:17) suitable for constructs according to the present invention)
ELKTPLGDTTHTEPKSCDTPPPCPRCP**GGGSSGGGSG**GQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK**GLGGL**

SEQ ID NO:14:

Hinge regions (IgG3 upper hinge), 12 amino acids: ELKTPLGDTTHT

SEQ ID NO:15:
Hinge region (IgG3, lower hinge, 15 amino acids): EPKSCDTPPPCPRCP

SEQ ID NO:16:
Gly-Ser Linker: GGGSSGGGSG

SEQ ID NO:17: hCH3 IgG3:
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK

SEQ ID NO:18: Linker: GLGGL

SEQ ID NO:19: Linker: GGGGSGGGGSGGGGS

SEQ ID NO:20: Upper hinge hIgG3 (h1): ELKTPLGDTTHT

SEQ ID NO:21: Lower hinge hIgG3 (h4): EPKSCDTPPPCPRCP

SEQ ID NO:22: CH3 hIgG3:

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKS
RWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK

SEQ ID NO:23: glycoprotein D from Herpes Simplex virus 2 with an optional His-tag in bold:

MGRLTSGVGTAALLVVAVGLRVVCAKYALADPSLKMADPNRFRGKNLPVLDQLTDPPGVKRVYHIQPSLEDPFQP
PSIPITVYYAVLERACRSVLLHAPSEAPQIVRGASDEARKHTYNLTIAWYRMGDNCAIPITVMEYTECPYNKSLG
VCPIRTQPRWSYYDSFSAVSEDNLGFLMHAPAFETAGTYLRLVKINDWTEITQFILEHRARASCKYALPLRIPPA
ACLTSKAYQQGVTVDSIGMLPRFIPENQRTVALYSLKIAGWHGPKPPYTSTLLPPELSDTTNATQPELVPEDPED
SALLEDPAGTVSSQIPPNWHIPSIQDVAPHHAPAAPSNPGLITG**HHHHHH**

SEQ ID NO:24: Amino acid sequence of suitable chicken vaccibody construct according to the invention: Targeting unit: anti-chicken MHC II scFv, dimerization unit: human shortened hinge (h1+h4), antigenic unit: gD from herpes simplex virus 2 + an optional 6xHis-tag in bold. (In N- to C-terminal order SEQ ID NO:11 + SEQ ID NO:14 + SEQ ID NO:15 + SEQ ID NO:16 + SEQ ID NO:17 + SEQ ID NO:18 + SEQ ID NO:23)

NIVMTQSPKSMSMSVGERVTLSCKASENVVTYVSWYQQKPDQSPKLLIYGASNRYTGVPDRFTGSGSATDFTLII
SSVQAEDLADYHCGQSYTYPPTFGAGTKLELK***GGGGSGGGGSGGGGS***EVKLVESGPSLVKPSQTLSLTCSVTGDS
ITSGYWNWIRKFPGKKLEYMGFISYSGDTYYNPSLKSRISITRDTSKNQYHLQLNSVTSEDTATYYCARRNYVSN
YGGWYFGVWGAGTTVTVSSELKTPLGDTTHTEPKSCDTPPPCPRCP***GGGSSGGGSG***GQPREPQVYTLPPSREEMT
KNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHN
RFTQKSLSLSPGK**GLGGL**MGRLTSGVGTAALLVVAVGLRVVCAKYALADPSLKMADPNRFRGKNLPVLDQLTDPP
GVKRVYHIQPSLEDPFQPPSIPITVYYAVLERACRSVLLHAPSEAPQIVRGASDEARKHTYNLTIAWYRMGDNCA
IPITVMEYTECPYNKSLGVCPIRTQPRWSYYDSFSAVSEDNLGFLMHAPAFETAGTYLRLVKINDWTEITQFILE
HRARASCKYALPLRIPPAACLTSKAYQQGVTVDSIGMLPRFIPENQRTVALYSLKIAGWHGPKPPYTSTLLPPEL
SDTTNATQPELVPEDPEDSALLEDPAGTVSSQIPPNWHIPSIQDVAPHHAPAAPSNPGLITG**HHHHHH**

Amino acid sequences of avian IgY CH2 and CH4 domains

SEQ ID NO:25: Chicken IgY CH2:

HPSSCTPSQSESVELLCLVTGFSPASAEVEWLVDGVGGLLVASQSPAVRSGSTYSLSSRVNVSGTDWREGKSYSC
RVRHPATNTVVEDHVKGCP

SEQ ID NO:26: Chicken IgY CH4:

GPTTPPLIYPFAPHPEELSLSRVTLSCLVRGFRPRDIEIRWLRDHRAVPATEFVTTAVLPEERTANGAGGDGDTF
FVYSKMSVETAKWNGGTVFACMAVHEALPMRFSQRTLQKQAGK

SEQ ID NO:27: Upper CH2 sequence IgY: EWLVDGVGGL

SEQ ID NO:28: Lower CH2 sequence IgY: EGKSYSCRVRHPATNTVVEDHVKGCP

SEQ ID NO:29: Extended lower CH2 sequence IgY: VSGTDWREGKSYSCRVRHPATNTVVEDHVKGCP

SEQ ID NO:30: Shortened HA5 with polybasic deletion from influenza A/VietNam 1203/04 H5N1:

DQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKKHNGKLCDLDGVKPLILRDCSVAGWLLGNPMCDE
FINVPEWSYIVEKANPVNDLCYPGDFNDYEELKHLLSRINHFEKIQIIPKSSWSSHEASLGVSSACPYQGK
SSFFRNVVWLIKKNSTYPTIKRSYNNTNQEDLLVLWGIHHPNDAAEQTKLYQNPTTYISVGTSTLNQRLVP
RIATRSKVNGQSGRMEFFWTILKPNDAINFESNGNFIAPEYAYKIVKKGDSTIMKSELEYGNCNTKCQTPM
GAINSSMPFHNIHPLTIGECPKYVKSNRLVLATGLRNSPQRERRRKKRGLFGAIAGFIEGGWQGMVDGW
YGYHHSNEQGSGYAADKESTQKAIDGVTNKVNSIIDKMNTQFEAVGREFNNLERRIENLNKKMEDGFLD
VWTYNAELLVLMENERTLDFHDSNVKNLYDKVRLQLRDNAKELGNGCFEFYHKCDNECMESVRNGTYDY
PQYSEEARLKREEISGVKLESIGIYQILSIYSTVASSL

SEQ ID NO:31: Amino acid sequence of suitable chicken vaccibody construct according to the invention:: Targeting unit: anti-chicken MHC II, dimerization unit: shortened CH2 (lower + upper CH2) + entire CH4, antigenic unit: H5 from influenza. (In N- to C-terminal order SEQ ID NO:11 + SEQ ID NO:27 + SEQ ID NO:28 + SEQ ID NO:16 + SEQ ID NO:26 + SEQ ID NO:18 + SEQ ID NO:30)

NIVMTQSPKSMSMSVGERVTLSCKASENVVTYVSWYQQKPDQSPKLLIYGASNRYTGVPDRFTGSGSAT
DFTLIISSVQAEDLADYHCGQSYTYPPTFGAGTKLELKGGGGSGGGGSGGGGSEVKLVESGPSLVKPSQT
LSLTCSVTGDSITSGYWNWIRKFPGKKLEYMGFISYSGDTYYNPSLKSRISITRDTSKNQYHLQLNSVTSE
DTATYYCARRNYVSNYGGWYFGVWGAGTTVTVSSEWLVDGVGGLEGKSYSCRVRHPATNTVVEDHVK
GCPGGGSSGGGSGGPTTPPLIYPFAPHPEELSLSRVTLSCLVRGFRPRDIEIRWLRDHRAVPATEFVTTAV
LPEERTANGAGGDGDTFFVYSKMSVETAKWNGGTVFACMAVHEALPMRFSQRTLQKQAGKGLGGLDQI
CIGYHANNSTEQVDTIMEKNVTVTHAQDILEKKHNGKLCDLDGVKPLILRDCSVAGWLLGNPMCDEFINV
PEWSYIVEKANPVNDLCYPGDFNDYEELKHLLSRINHFEKIQIIPKSSWSSHEASLGVSSACPYQGKSSFF
RNVVWLIKKNSTYPTIKRSYNNTNQEDLLVLWGIHHPNDAAEQTKLYQNPTTYISVGTSTLNQRLVPRIAT
RSKVNGQSGRMEFFWTILKPNDAINFESNGNFIAPEYAYKIVKKGDSTIMKSELEYGNCNTKCQTPMGAI
NSSMPFHNIHPLTIGECPKYVKSNRLVLATGLRNSPQRERRRKKRGLFGAIAGFIEGGWQGMVDGWYGY
HHSNEQGSGYAADKESTQKAIDGVTNKVNSIIDKMNTQFEAVGREFNNLERRIENLNKKMEDGFLDVWT
YNAELLVLMENERTLDFHDSNVKNLYDKVRLQLRDNAKELGNGCFEFYHKCDNECMESVRNGTYDYPQY
SEEARLKREEISGVKLESIGIYQILSIYSTVASSL

SEQ ID NO:32: Variant 2 with an extended lower CH2 sequence:
(In N- to C-terminal order SEQ ID NO:11 + SEQ ID NO:29 + SEQ ID NO:16 + SEQ ID NO:26 + SEQ ID NO:18 + SEQ ID NO:30)

NIVMTQSPKSMSMSVGERVTLSCKASENVVTYVSWYQQKPDQSPKLLIYGASNRYTGVPDRFTGSGSAT
DFTLIISSVQAEDLADYHCGQSYTYPPTFGAGTKLELKGGGGSGGGGSGGGGSEVKLVESGPSLVKPSQT
LSLTCSVTGDSITSGYWNWIRKFPGKKLEYMGFISYSGDTYYNPSLKSRISITRDTSKNQYHLQLNSVTSE
DTATYYCARRNYVSNYGGWYFGVWGAGTTVTVSSVSGTDWREGKSYSCRVRHPATNTVVEDHVKGCP
GGGSSGGGSGGPTTPPLIYPFAPHPEELSLSRVTLSCLVRGFRPRDIEIRWLRDHRAVPATEFVTTAVLPEE
RTANGAGGDGDTFFVYSKMSVETAKWNGGTVFACMAVHEALPMRFSQRTLQKQAGKGLGGLDQICIGY
HANNSTEQVDTIMEKNVTVTHAQDILEKKHNGKLCDLDGVKPLILRDCSVAGWLLGNPMCDEFINVPEW
SYIVEKANPVNDLCYPGDFNDYEELKHLLSRINHFEKIQIIPKSSWSSHEASLGVSSACPYQGKSSFFRNV
VWLIKKNSTYPTIKRSYNNTNQEDLLVLWGIHHPNDAAEQTKLYQNPTTYISVGTSTLNQRLVPRIATRSK
VNGQSGRMEFFWTILKPNDAINFESNGNFIAPEYAYKIVKKGDSTIMKSELEYGNCNTKCQTPMGAINSS
MPFHNIHPLTIGECPKYVKSNRLVLATGLRNSPQRERRRKKRGLFGAIAGFIEGGWQGMVDGWYGYHHS
NEQGSGYAADKESTQKAIDGVTNKVNSIIDKMNTQFEAVGREFNNLERRIENLNKKMEDGFLDVWTYNA
ELLVLMENERTLDFHDSNVKNLYDKVRLQLRDNAKELGNGCFEFYHKCDNECMESVRNGTYDYPQYSEE
ARLKREEISGVKLESIGIYQILSIYSTVASSL

SEQ ID NO:33: Infectious bursal disease virus viral protein 2, viral protein 4 and viral protein 3 of segment A (VP2-VP4-VP3); References: 1) De Schutter K., Lin Y.-C., Tiels P, et al. (2009). "Genome sequence of the recombinant protein production host Pichia pastoris.". Nature Biotechnology 27 (6): 561–566, 2) Daly R, Hearn MT (2005). "Expression of heterologous proteins in Pichia pastoris: a useful experimental tool in protein engineering and production". J. Mol. Recognit. 18 (2): 119–38, 3) Pitcovski J., Gutter B., Gallili G., et al. (2003). "Development and large-scale use of recombinant VP2 vaccine for the prevention of infectious bursal disease of chickens". Vaccine 21 4736-43, DNA Sequence:

ATGACAAACCTGCAAGATCAAACCCAACAGATTGTTCCGTTCATACGGAGCCTTCTGATGCCAACAACCGGACCGGCGT
CCATTCCGGACGACACCCTAGAGAAGCACACTCTCAGGTCAGAGACCTCGACCTACAATTTGACTGTGGGGGACACAGG
GTCAGGGCTAATTGTCTTTTTCCCTGGTTTCCCTGGCTCAATTGTGGGTGCTCACTACACACTGCAGAGCAATGGGAAC
TACAAGTTCGATCAGATGCTCCTGACTGCCCAGAACCTACCGGCCAGCTACAACTACTGCAGGCTAGTGAGTCGGAGTC
TCACAGTGAGGTCAAGCACACTCCCTGGTGGCGTTTATGCACTAAATGGCACCATAAACGCCGTGACCTTCCAAGGAAG
CCTGAGTGAACTGACAGATGTTAGCTACAATGGGTTGATGTCTGCAACAGCCAACATCAACGACAAAATCGGGAACGTC
CTAGTAGGGGAAGGGGTAACCGTCCTCAGCTTACCCACATCATATGATCTTGGGTATGTGAGACTCGGTGACCCCATTC
CCGCTATAGGGCTCGACCCAAAAATGGTAGCAACATGTGACAGCAGTGACAGGCCCAGAGTCTACACCATAACTGCAGC
CGATGATTACCAATTCTCATCACAGTACCAGGCAGGTGGGGTAACAATCACACTGTTCTCAGCTAATATCGATGCCATC
ACAAGCCTCAGCATTGGGGGAGAACTCGTGTTCCAAACAAGCGTCCAAGGCCTTATACTGGGTGCTACCATCTACCTTA
TAGGCTTTGATGGGACTGCGGTAATCACCAGAGCTGTGGCCGCAGACAATGGGCTAACGGCCGGCACTGACAACCTTAT
GCCATTCAATATTGTGATTCCAACCAGCGAGATAACCCAGCCAATCACATCCATCAAACTGGAGATAGTGACCTCCAAA
AGTGGTGGTCAGGCGGGGGACCAGATGTCATGGTCAGCAAGTGGGAGCCTAGCAGTGACGATCCACGGTGGCAACTATC
CAGGGGCCCTCCGTCCCGTCACACTAGTAGCCTACGAAAGAGTGGCAACAGGATCTGTCGTTACGGTCGCCGGGGTGAG

CAACTTCGAGCTGATCCCAAATCCTGAACTAGCAAAGAACCTGGTCACAGAATACGGCCGATTTGACCCAGGAGCCATG
AACTACACAAAATTGATACTGAGTGAGAGGGACCGTCTTGGCATCAAGACCGTATGGCCAACAAGGGAGTACACTGACT
TTCGCGAGTACTTCATGGAGGTGGCCGACCTCAACTCTCCCCTGAAGATTGCAGGAGCATTTGGCTTCAAAGACATAAT
CCGGGCCATAAGGAGGATAGCTGTGCCGGTGGTCTCTACATTGTTCCCACCCGCCGCTCCCCTAGCCCATGCAATTGGG
GAAGGTGTAGACTACCTGCTGGGCGATGAGGCACAGGCTGCTTCAGGAACTGCTCGAGCCGCGTCAGGAAAAGCAAGAG
CTGCCTCAGGCCGCATAAGGCAGCTAACTCTCGCCGCCGACAAGGGGTACGAGGTAGTCGCGAATCTGTTTCAGGTGCC
CCAGAATCCTGTAGTCGACGGGATTCTCGCTTCACCTGGGATACTCCGCGGTGCACACAACCTCGACTGCGTGTTGAGA
GAGGGTGCCACGCTATTCCCTGTGGTCATCACGACAGTGGAAGATGCCATGACACCCAAAGCATTGAACAGCAAAATGT
TTGCTGTCATTGAAGGCGTGCGAGAAGATCTACAACCTCCATCTCAAAGAGGATCCTTCATACGAACTCTCTCCGCACA
TAGAGTCTATGGATATGCTCCAGATGGGGTACTTCCACTGGAGACTGGGAGAGATTACACCGTGGTCCCAATAGATGAT
GTCTGGGATGACAGCATTATGCTGTCCAAAGACCCCATACCTCCTATTGTGGGAAACAGTAGAAACCTAGACATAGCTT
ACATGGATGTGTTTCGACCCAAAGTCCCCATCCATGTGGCCATGACGGGAGCCCTCAATGCCTATGGCGAGATTGAGAA
CGTGAGCTTTAGAAGCACCAAGCTCGCCACTGCACACCGACTTGGCCTCAAGTTGGCTGGTCCCGGTGCATTTGACGTG
AACACCGGGTCCAACTGGGCAACGTTCATCAAACGTTTCCCTCACAATCCACGAGACTGGGACAGGCTCCCTTACCTCA
ACCTTCCATACCTTCCACCCAATGCAGGACGCCAGTACGACCTGGCCATGGCTGCTTCAGAGTTCAAAGAGACCCCCGA
ACTCGAGAGCGCCGTCAGAGCCATGGAAGCAGAAGCCAACGTGGACCCACTGTTCCAATCTGCACTCAGCGTGTTCATG
TGGCTGGAAGAGAATGGGATTGTGACTGATATGGCCAACTTCGCACTCAGCGACCCGAATGCCCATCGGATGCGCAATT
TTCTCGCAAACGCACCACAAGCAGGCAGCAAGTCGCAAAGAGCCAAGTACGGGACAGCAGGCTACGGAGTGGAGGCCCG
GGGCCCCACTCCAGAGGAAGCACAGAGGGAAAAAGACACACGGATCTCAAAGAAGATGGAGACTATGGGCATCTACTTT
GCAACACCAGAATGGGTAGCACTCAATGGGCACCGGGGGTCAAGCCCCGGCCAGCTAAAGTACTGGCAGAACACACGAG
AAATACCTGATCCAAACGAGGACTACCTAGACTACGTGCATGCAGAGAAGAGCCGGTTGGCATCAGAAGAACAAATCCT
AAGGGCAGCCACGTCGATCTACGGGGCTCCAGGACAGGCTGAGCCACCCCAGGCCTTCATAGACGAAGTCGCCAAAGTC
TATGAAATCAACCATGGGCGTGGCCCCAACCAAGAACAGATGAAAGATCTGCTCTTGACTGCGATGGAGATGAAGCATC
GCAATCCCAGGCGGGCTCCACCAAAGCCCAAGCCAAAACCCAATGTTCCAACACAGAGACCCCCTGGTCGGCTGGGCCG
CTGGATCAGGGCTGTCTCTGATGAGGACCTTGAGTGA

SEQ ID NO:34: Amino Acid sequence:

MTNLQDQTQQIVPFIRSLLMPTTGPASIPDDTLEKHTLRSETSTYNLTVGDTGSGLIVFFPGFPGSIVGAHYTLQSNGN
YKFDQMLLTAQNLPASYNYCRLVSRSLTVRSSTLPGGVYALNGTINAVTFQGSLSELTDVSYNGLMSATANINDKIGNV
LVGEGVTVLSLPTSYDLGYVRLGDPIPAIGLDPKMVATCDSSDRPRVYTITAADDYQFSSQYQAGGVTITLFSANIDAI
TSLSIGGELVFQTSVQGLILGATIYLIGFDGTAVITRAVAANNGLTAGTDNLMPFNIVIPTSEITQPITSIKLEIVTSK
SGGQAGDQMSWSASGSLAVTIHGGNYPGALRPVTLVAYERVATGSVVTVAGVSNFELIPNPELAKNLVTEYGRFDPGAM
NYTKLILSERDRLGIKTVWPTREYTDFREYFMEVADLNSPLKIAGAFGFKDIIRALRRIAVPVVSTLFPPAAPL

SEQ ID NO:35: Influenza A virus (A/chicken/Israel/215/2007(H9N2)) segment 4 hemagglutinin (HA); Reference: GenBank: FJ464716.1, DNA sequence:

```
   1 atggaaataa tatcactgat gactatacta ctagtagtaa caacaagcaa tgcagataaa
  61 atatgcattg gccaccagtc aacaaattcc acagaaactg tggatacact aacagaaact
 121 aatgttcctg tgacacatgc caaagaattg ctccacacag agcacaatgg aatgctgtgt
 181 gcaacaaatc tgggaaatcc cctcatccta gacacatgca ctatcgaagg acttatctat
```

```
 241 ggtaaccctt cttgtgacat gttgttgggg ggaagggaat ggtcctacat cgttgaaaga
 301 ccatcagcgg taaatggaac atgttaccct gggaatgtgg aaaacttaga ggaactcaga
 361 acacttttta gttcctctag ttcctatcaa agaatccaaa tattcccaga cacaatctgg
 421 aatgtgactt acaatggaac aagcaaatca tgttcaaatt cattctacag gaatatgaga
 481 tggctaactc aaaagaacgg ggtttatcct gttcaagacg cccaatacac aaataatcgg
 541 ggaaaggaca ttcttttcgt gtggggcata catcatccac ccaccgatac tgcacagacg
 601 aatttgtaca caagaaccga cacaacaaca agcgtaacaa cagaaaattt agataggacc
 661 ttcaaaccat tgatagggcc aaggcccctt gtcaatggtc tgattggaag aattaattat
 721 tattggtcgg tactaaaacc aggccagaca ttgcgagtaa gatccaatgg gaatctaatt
 781 gctccatggt ttggacacgt tctctcagga gagagccatg ggagaatcct gaaaactgat
 841 ttaaacagtg gtaattgtgt agtgcaatgt cagactgaaa aaggtggcct aaacagtaca
 901 ttgcctttcc acaatatcag taaatatgca tttgggaatt gccccaaata tattggagtc
 961 aagagtctca aactggcaat cggtctgaga aacgtgcctg ccaggtcaag tagaggacta
1021 tttggagcca tagctggatt catagaagga ggttggccag ggctagtcgc cggttggtat
1081 ggtttccagc attcaaatga tcaaggggtt ggtatggctg cagataggga ttcaactcaa
1141 aaggcagttg acaaaataac atccaaggtg aataatatag tcgacaagat gaacaagcaa
1201 tatgaaataa ttgatcatga attcagtgag gttgaaacta gactcaatat gatcaataac
1261 aagattgatg accaaataca agatgtatgg gcatataatg cagagttgct agtactactt
1321 gagaaccaga aaacactcga tgagcatgac gcaaacgtga acaacctata taacaaggtg
1381 aaaagggcct tgggctccaa tgcgatggaa gatgggaagg gctgtttcga gctataccac
1441 aaatgtgatg accaatgcat ggaaactatt cggaacggga cctataatag gagaaagtac
1501 aaagaggaat caagactaga aaggcagaaa atagagggag tcaaactgga atctgagggg
1561 atttacaaaa tacttaccat ttattcgact gtcgcctcat ctcttgtact tgcaatgggg
1621 tttgctgcct tcttattctg ggccatgtcc aatggatcat gcaggtgcaa catctgtata
1681 taattagcaa aaacaccctt gtttcta
```

SEQ ID NO:36: Amino acid sequence:

```
MEIISLMTILLVVTTSNADKICIGHQSTNSTETVDTLTETNVPVTHAKELLHTEHNGMLCATNLGNPLILDTCTIEGLI
YGNPSCDMLLGGREWSYIVERPSAVNGTCYPGNVENLEELRTLFSSSSSYQRIQIFPDTIWNVTYNGTSKSCSNSFYRN
MRWLTQKNGVYPVQDAQYTNNRGKDILFVWGIHHPPTDTAQTNLYTRTDTTTSVTTENLD
RTFKPLIGPRPLVNGLIGRINYYWSVLKPGQTLRVRSNGNLIAPWFGHVLSGESHGRI
LKTDLNSGNCVVQCQTEKGGLNSTLPFHNISKYAFGNCPKYIGVKSLKLAIGLRNVPA
RSSRGLFGAIAGFIEGGWPGLVAGWYGFQHSNDQGVGMAADRDSTQKAVDKITSKVNN
IVDKMNKQYEIIDHEFSEVETRLNMINNKIDDQIQDVWAYNAELLVLLENQKTLDEHDANVNNLYNKVKRALGSNAMED
GKGCFELYHKCDDQCMETIRNGTYNRRKYKEESRLER
QKIEGVKLESEGIYKILTIYSTVASSLVLAMGFAAFLFWAMSNGSCRCNICI
```

SEQ ID NO:37: Avian infectious bronchitis virus isolate variant 1 S1 spike glycoprotein gene, Reference: GenBank: AF093795.1; DNA sequence:

```
   1 atgttgggca aaccgctttt actagtgact ctttggtatg cactatgtag tgctttgctc
```

```
  61 tatgatagta gtacttacgt ttactactac caaagtgctt ttaggcctag ttcaggttgg
 121 cacatacatg ggggtgctta tgcagtagat agggttttta atgaaaccaa caatgcaggc
 181 agtgtatctg attgcactgc tggtactttt tatgaaagcc ataatatttc tgctgtttct
 241 gtagccatga cagcaccaca taatggtatg tcttggtcag tttcacaatt ttgtacagct
 301 cattgtaact tctcagactt tacagtgttc gttacgcatt gttttaaaaa tcaacctggt
 361 agttgtccct tgacaggtat gattcctcag aatcatattc gtatttctgc tatgagacaa
 421 ggaactttgt tttataactt aacagttagt gtgtctaaat atcctagatt taaatcgctt
 481 caatgtgtta gcaattctac atctgtctat gtaaatggtg atcttgtttt cacttctaat
 541 gaaacttctt acattacggg tgcaggcgtt tattttaaaa gtggtgggcc tgtaacttat
 601 aaagttatga aagaagttaa agccctagcc tactttatta atggtaccgc acaagaggtt
 661 attttatgtg ataactcacc tagaggtttg cttgcatgtc agtataatac tggtaatttt
 721 tcagatggat tctacccttt tactaatcat tctttagtta aggataggtt tattgtatat
 781 cgagaaagtg gcactaacac tactttaaag ttaactaatt tcagttttac taatgtaagt
 841 aatgctcctc ctaattcagg tggcgttgat actttccaat tatatcaaac acatactgct
 901 caggatggtt attataattt taatttatca tttctgagta gttttgtgta taaaccatct
 961 gattttatgt atgggtcata ccacccacat tgtaatttta gaccagaaaa tattaataat
1021 ggcttatggt ttaattcatt atctgtgtca cttacttacg gacccattca aggtggttgt
1081 aagcaatctg tttttaataa tagagcaact tgttgctatg cttattctta tcaagggcct
1141 agtttatgta agggtgttta tagaggggag ctaatgcaat actttgaatg tggacttcta
1201 gtttacgtaa ctaagagtga tggctctcgt atacaaacta gaagtgaacc acttgtgtta
1261 actcaatata attataacaa cattacttta aacaagtgtg ttgagtataa tatatatggt
1321 agagttggtc aaggtcttat tactaatgta actgaagcaa ctgctaatta tagttatcta
1381 gcagatggtg gtttagctat tttagatacc tcaggagcca tagacatatt tgttgttcaa
1441 ggtgcacatg gtcttaatta ttataaggtt aatccctgtg aggatgttaa ccaacagttt
1501 gtagtgtcag gtggcaactt agttggcatt cttacatctc ataatgaaac aggttctgaa
1561 tctattgaga accagtttta catcaaactc actaacggaa cacgtcgctc tagacgt
```

SEQ ID NO:38: Amino acid sequence:

```
MLGKPLLLVTLWYALCSALLYDSSTYVYYYQSAFRPSSGWHIHGGAYAVDRVFNETNNAGSVSDCTAGTFYESHNISAV
SVAMTAPHNGMSWSVSQFCTAHCNFSDFTVFVTHCFKNQPGSCPLTGMIPQNHIRISAMRQGTLFYNLTVSVSKYPRFK
SLQCVSNSTSVYVNGDLVFTSNETSYITGAGVYFKSGGPVTYKVMKEVKALAYFINGTAQEVILCDNSPRGLLACQYNT
GNFSDGFYPFTNHSLVKDRFIVYRESGTNTTLKLTNFSFTNVSNAPPNSGGVDTFQLYQTHTAQDGYYNFNLSFLSSFV
YKPSDFMYGSYHPHCNFRPENINNGLWFNSLSVSLTYGPIQGGCKQSVFNNRATCCYAYSYQGPSLCKGVYRGELMQYF
ECGLLVYVTKSDGSRIQTRSEPLVLTQYNYNNITLNKCVEYNIYGRVGQGLITNVTEATANYSYLADGGLAILDTSGAI
DIFVVQGAHGLNYYKVNPCEDVNQQFVVSGGNLVGILTSHNETGSESIENQFYIKLTNGTRRSRR
```

SEQ ID NO:39: Eimeria maxima 56 kDa gametocyte antigen (gam56) Reference: GenBank: AY129951.2; DNA sequence:

```
   1 agcagaacat agggagttca tctgttcctt cttttcatca tttattcctc gtttctcacc
  61 gttttatttt ttttgtgtaa ccctctccgc tgttgagtcc caatgacccg cctcggcctc
```

```
 121 gctgctgtcg cgctggctct cgccgtgggc ccttccatgg cagtgcccag caccactcct
 181 gttgagaacc aggttcaccc ttacagcgag atgagtacct accaggaggg gagtgccccg
 241 ggggctccgg aggacaccac caccaccact acgtcgtccc ctgtttccga tggagccgag
 301 cagtggcttg agagctttgt tcgtgctgtg cagcgccagc tgcagcttca ggaccaaatg
 361 atgcgtcagc tcatgaggga cattcaggag tacctgagca ctgcgttcaa ctgggcagag
 421 aaccagtcta ctgcctacac ccgtgttacc gagatgatgg acatgatctc caacagaatg
 481 aacgctgcca tggacagctc aaacgaactc atgaccacta gcgacaccac agaccccgag
 541 accctccgcc gtgcaactcg caagtacatg aaggaggttc gcgttcagga cgtcctggta
 601 gatgctctct gggcctctct ccgcggtgta cagacagctg cctggatgaa tggagtgacc
 661 gctattgaga aggaggagac gactcccatg gctagccgcg ctgctgagga gttcctccac
 721 cgcatgtacc ataacctgag ggcagcaggt atgtctgaag aagatgttgc caagttcatc
 781 cctagagccg agtacaaccc ctccgagcag tcaagaaata tgggcagaaa gggcaggagc
 841 ttctactacg gcggctatcc cagctactac aactcccccc actacagcta cagcagctac
 901 cccagctact acaactacag ctacccgtca tacagctaca gcagctaccc cagctactac
 961 cgctacagca gctaccccta ctacaactac agctatccca gctactacaa ctacggcagc
1021 taccctact acagttatag cagctacccc agctggtact ggcgccgtct ccgctctttg
1081 gcaacagcaa cttgcccaga ctgccctcct ctcaccactc ccagcatgat cccaactccc
1141 cccccaatga tgaacatgat gaacacccca ccccccatgg caaacatgat gaccagcatg
1201 atgatgaaca ctcccatggt tcctcctccc cgcaccctcg gaactgaagc catgagcctc
1261 ggcttggccc ccatcggtat caccggcgcc cccatgacag gtttcggtgt tcctcctgag
1321 ttcggtccct ttggagccga aggtatcggc ctccccaccg atgccctcgg cagcaccccc
1381 gaaatgacac cattcgaccc aactaccccc tacagaactc tcgcccccat ggacctcccc
1441 cccatccccc ctcctgtctt ccctgaaacc cctatgaggc cacctactcc cttcggcttc
1501 ggacctgcac ctgttcctcc catgcccttc taaacgacct accatccctc aatccatagc
1561 tcacatttcg tagcctcaaa acagtttttt gttcatttca cttccaggac tcatgctgcg
1621 acatttgcat tcgtacctcg aaaccgtcaa cctcaaaccc caaaccattc tgtgacctcc
1681 cctcgcaaac gcggaaggcg gaacattttt tctgaagtat attactacgt taaaaaaaaa
1741 aaaaaaaaaa aaaa
```

SEQ ID NO:40: Amino acid sequence:

```
MTRLGLAAVALALAVGPSMAVPSTTPVENQVHPYSEMSTYQEGSAPGAPEDTTTTTTSSPVSDGAEQWLESFVRAVQRQ
LQLQDQMMRQLMRDIQEYLSTAFNWAENQSTAYTRVTEMMDMISNRMNAAMDSSNELMTTSDTTDPETLRRATRKYMKE
VRVQDVLVDALWASLRGVQTAAWMNGVTAIEKEETTPMASRAAEEFLHRMYHNLRAAGMSEEDVAKFIPRAEYNPSEQS
RNMGRKGRSFYYGGYPSYYNSPYYSYSSYPSYYNYSYPSYSYSSYPSYYRYSSYPYYNYSYPSYYNYGSYPYYSYSSYP
SWYWRRLRSLATATCPDCPPLTTPSMIPTPPPMMNMMNTPPPMANMMTSMMMNTPMVPPPRTLGTEAMSLGLAPIGITG
APMTGFGVPPEFGPFGAEGIGLPTDALGSTPEMTPFDPTTPYRTLAPMDLPPIPPPVFPETPMRPPTPFGFGPAPVPPM
PF
```

EXAMPLES

EXAMPLE 1:
Construction of 2G11-vaccibody

For utilising the antibody 2G11 as a targeting unit in a vaccibody vaccine, the coding region of the antibody constituting the antigen-binding site must be isolated, verified and cloned into the vaccibody format. The antigen binding parts of antibodies are the variable domains composed of a heavy and a light chain. These domains are denoted VH and VL, respectively. In the vaccibody format two amino acid chains are folded into a dimer. Therefore, the desired form of a VH and VL antibody fragment is a ScFv fragment which is composed of the VL and VH combined through a flexible linker.

mRNA, cDNA synthesis and RT PCR of 2G11 VH and VL genes:

Oligonucleotide sequences:

IgκcDNAprimer: (atcaggacagcaaagacagca) TGCTGTCTTTGCTGTCCTGAT (SEQ ID NO:1)

3´IgκC rev: (CAAGAAGCACACGACTGAGGC) gcctcagtcgtgtgcttcttg (SEQ ID NO:4)

PolyG_NotI_frwd: ATATGCGGCCGCGGGGGGGGGGGGGGG (SEQ ID NO:2)

3´-mIgG1rev: ttg acc agg cat ccc agg gtc (SEQ ID NO:3)

VHL1: ggt gtg cat tcc atg gac tgg acc tgg agg (SEQ ID NO:5)

RNA was isolated from the MaD2G11 hybridoma (Salomonsen et.al. Immunogenetics 1987; 25(6):373-82) cells, by using the Absolutely RNA® Miniprep Kit (Stratagene). The cDNA synthesis was performed according to protocol using the IgκcDNAprimer (SEQ ID NO:1) and poly dCTP 3`-tailing of the cDNA using terminal transferase was performed by mixing 10 µl cDNA (unknown concentration), 2µl 1 x TdT reaction buffer, 2µl $CoCl_2$, 1µl dCTP, 2µl rTdT and 4µl ddH_2O and incubating the mixture for 15 min at 37°C. Then the solution was placed on ice, glycogen precipitated as in step 5 and rehydrated in 20µl dH_2O on ice (see e.g. Nilssen NR et al. Nucleic Acids Res. 2012 Sep;40(16):e120). PCR reaction for amplification of variable light (VL) genes was performed by using the oligonucleotides PolyG NotI frwd (SEQ ID NO:2) and oligonucleotides compatible to mouse Constant Kappa (SEQ ID NO:4) , while

the variable heavy (VH) genes was synthesized using oligonucleotides compatible with the VH leader (SEQ ID NO:5) and mouse IgG1 constant region (SEQ ID NO:3) sequences.

The PCR products were further cloned into vectors by TOPO-cloning according to the manufacturers description (Zero Blunt® TOPO PCR Cloning Kit, Invitrogen).

Verification of 2G11 VH and VL sequences:

Seven isolated plasmids form each of the VL and VH cloning procedures were sequenced and productive VL and VH sequences were verified by homology search (IMGT/V QUEST).

SEQ ID NO:6: 2G11 VH nucleotide sequence:
gaggtgaagctggtggagtcaggacctagcctcgtgaaaccttctcagactctgtccctc
acctgttctgtcacgggcgactccatcaccagtggttattggaactggatccggaaattc
ccagggaaaaaacttgaatacatggggttcataagctacagtggtgacacttattacaat
ccatctctcaaaagtcgaatctccatcactcgagacacatccaagaaccagtaccacctg
cagttgaattctgtgacttctgaggacacagcaacatattactgtgcaagaaggaactac
gttagtaactacgggggggtggtacttcggtgtctggggcgcagggaccacggtcaccgtc
tcctca

SEQ ID NO:7: 2G11 VH amino acid sequence:
EVKLVESGPSLVKPSQTLSLTCSVTGDSITSGYWNWIRKFPGKKLEYMGFISYSGDTYYN
PSLKSRISITRDTSKNQYHLQLNSVTSEDTATYYCARRNYVSNYGGWYFGVWGAGTTVTV
SS

SEQ ID NO:8: 2G11 VL nucleotide sequence:
aacattgtaatgacccaatctcccaaatccatgtccatgtcagtaggagagagagtcacc
ctgagctgcaaggccagtgagaatgtggttacttatgtatcctggtatcaacagaaacca
gatcagtctcctaaactgctgatatacggggcatccaaccggtacactggggtccctgat
cgcttcacaggcagtggatctgcaacagatttcactcttatcatcagcagtgttcaggct
gaagaccttgcagattatcactgtggacagagttacacctatcctcccacgttcggtgct
gggaccaagctggagctgaaa

SEQ ID NO:9: 2G11 VL amino acid sequence:
NIVMTQSPKSMSMSVGERVTLSCKASENVVTYVSWYQQKPDQSPKLLIYGASNRYTGVPD
RFTGSGSATDFTLIISSVQAEDLADYHCGQSYTYPPTFGAGTKLELK

Design of 2G11 single chain Fv (ScFv) construct :

The following ScFv was constructed.

SEQ ID NO:33:2G11 ScFv nucleotide sequence including restriction enzyme sites:

tgcattccaacattgtaatgacccaatctcccaaatccatgtccatgtcagtagga
gagagagtcaccctgagctgcaaggccagtgagaatgtggttacttatgtatcctggtat

caacagaaaccagatcagtctcctaaactgctgatatacggggcatccaaccggtacact
ggggtccctgatcgcttcacaggcagtggatctgcaacagatttcactcttatcatcagc
agtgttcaggctgaagaccttgcagattatcactgtggacagagttacacctatcctccc
acgttcggtgctgggaccaagctggagctgaaaggtggaggcggatctggcggaggtggc
tctggcggtggcggatcggaggtgaagctggtggagtcaggacctagcctcgtgaaacct
tctcagactctgtccctcacctgttctgtcacgggcgactccatcaccagtggttattgg
aactggatccggaaattcccagggaaaaaacttgaatacatggggttcataagctacagt
ggtgacacttattacaatccatctctcaaaagtcgaatctccatcactcgagacacatcc
aagaaccagtaccacctgcagttgaattctgtgacttctgaggacacagcaacatattac
tgtgcaagaaggaactacgttagtaactacgggggggtggtacttcggtgtctggggcgca
gggaccacggtcaccgtctcctcaggtgagtcgtacg

SEQ ID NO:11: 2G11 ScFv coding aminoacid sequence:
NIVMTQSPKSMSMSVGERVTLSCKASENVVTYVSWYQQKPDQSPKLLIYGASNRYTGVPDRFTGSGSAT
DFTLIISSVQAEDLADYHCGQSYTYPPTFGAGTKLELKGGGGSGGGGSGGGGSEVKLVESGPSLVKPSQT
LSLTCSVTGDSITSGYWNWIRKFPGKKLEYMGFISYSGDTYYNPSLKSRISITRDTSKNQYHLQLNSVTSE
DTATYYCARRNYVSNYGGWYFGVWGAGTTVTVSS

Construction of 2G11-vaccibody:

The 2G11 scFv construct (SEQ ID NO:33) was cloned into the plasmid pLNOH2 (Norderhaug, L. et al., J Immunol Methods, 1997) encoding a vaccibody framework at BsmI and BsiWI sites, giving the overall vaccibody format: 2G11 scFv-dimerisation domain-antigen. The dimerization domain was a human hinge region and CH3 as described in e.g. the International Patent Application with application number PCT/EP2012/076404 and the antigenic part was derived from Herpes Simplex virus 2, gD protein (SEQ ID NO:23). The construct also encoded a His-tag for easy detection in ELISA.

EXAMPLE 2:

Verification of 2G11-vaccibody targeting Chicken APCs

For the purpose of utilising 2G11 in a vaccibody vaccine directed towards chicken diseases, a prerequisite is that the novel vaccibody protein is able to bind chicken antigen presenting cells. 2G11 is recognising chicken MHCII. However, after isolation of only the Fv part of an intact antibody and transferred to a novel format, a verification of the sustainability of specificity is mandatory. The following example shows how the 2G11 vaccibody was produced as a protein and analysed for binding to chicken antigen presenting cells by flow cytometry.

Transfection and protein production and purification:

HEK293E cells were transiently transfected with the pLNOH2 2G11-vaccibody construct by using Lipofectamine™ 2000 (Invitrogen). Culture medium was harvested at day 3 and 6 and further concentrated by the use of Vivaspin 2 columns. The concentrated medium was tested by ELISA for protein production. Shortly, immunoplates were coated with anti-human CH3

antibody (MCA878, AbD Serotec). Dilutions of culture medium from transfected and un-transfected cells were added. 2G11-vaccibody proteins were further detected by adding anti-His tag antibody (ab27025, Abcam).

Isolation and staining of chicken PBMCs with 2G11-vaccibody:

PBMCs from 24ml chicken blood was isolated with Lymphoprep (Lympholyte®-M, Cedarlane) and the cells were adjusted to a final concentration of $10x10^6$cells/ml. The cells were resuspended in 100µl 10µg/ml Fc Block and incubated for 15 min at 4 ºC. The cells were further stained for MHC II binding by adding 25 µl concentrated 2G11 vaccibodies followed by 10µg/ml biotinylated anti-human IgG (05-4240, Invitrogen) and streptavidin-PE or corresponding isotype controls. A positive control was stained with 10µg/ml 2G11 mAb (AH Diagnostics) and a negative medium control was included.

The cells were analyzed on a BD FACSCalibur by using the software CellQuest by gating for live lymphocytes, CD45 APC positive. The following histogram analysis is for MHC binding to CD45+ cells.

There is strong, clear binding of both the 2G11 mAb and 2G11 vaccibody to CD45+ leukocytes from chicken blood. Surprisingly the 2G11 vaccibody seems to bind the chicken cells better than the native 2G11 mAb. There is no binding of the negative controls; staining buffer, the non-targeting vaccibody or the isotype controls. The example verifies that 2G11 vaccibodies are able to target chicken antigen presenting cells.

EXAMPLE 3.

Immune response studies

Constructs according to the present invention are selected as vaccine candidates with their corresponding controls. As a negative control empty vector is utilized.

Different amounts of plasmid DNA of each candidate is administered by bodily injection, in the drinking water, as a spray for inhalation or injected in ovo. Chicken blood is drawn every week after vaccination for measurement of antigen specific antibodies. The antibody responses are calculated by ELISA.

EXAMPLE 4.

Preventive effect

Chicken vaccinated with selected vaccibody vaccine candidates are challenged with pathological levels of the corresponding pathogen, being a virus or bacteria. Challenged chickens are monitored for disease development. The monitoring performed by measuring

virus or bacterial levels as well as disease progression associated with the respective disease condition.

EXAMPLE 5.

A DNA vaccine to be used may be prepared by GMP manufacturing of the plasmid vaccine according to regulatory authorities' guidelines, including GMP cell banking, GMP manufacturing of drug substance and drug product, ICH stability studies and Fill & Finish of the DNA vaccine. The DNA vaccine may be formulated by dissolving in a saline solution, such as 10nM Tris, 1mM EDTA or PBS pH7,4 at a concentration of 1-3 mg/ml or included in suitable viral vector systems. The vaccine may be administered either intra-dermal or intra-muscular, respiratorial, mucosal or via the GI tract, or in ovo.

EXAMPLE 6.

The scFv fragment constituting the targeting unit of the described invention may be manipulated by means of altering affinity and specificity. The 2G11 scFv clone may be displayed as a fusion to phage particles and variations in the CDR- regions can be introduced by either erroneous PCR or specific PCR reactions with oligonucleotides introducing heterogeneity in the CDR regions. After generating a phage display library specific clones can be selected towards the specific target, i.e. a chicken MHC class II molecule. The selection process can be performed at different stringencies, such as low target concentration, high temperature or altered salt concentrations. Such conditions may develop a 2G11 scFv fragment with higher specificity and affinity towards the specific chicken MHCII molecule. Likewise, to obtain a 2G11 scFv fragment with a broader specificity, the phage display library can be selected towards a variety of MCHII molecules and selected clones harbouring specificity towards a variety of MHCII molecules can thus be selected. The manipulated scFV fragments can be utilised as new targeting units of the described invention.

CLAIMS

1. A homodimeric protein of two identical amino acid chains, each amino acid chain comprising (1) optionally a signal peptide, (2) a targeting unit, (3) a dimerization motif, and (4) an antigenic unit, said targeting unit being a scFv fragment specifically binding avian MHC class II molecule, such as chicken MHC class II molecule, on avian cells.

2. The homodimeric protein according to claim 1, said targeting unit comprising an amino acid sequence having at least 80 % sequence identity to the amino acid sequence of SEQ ID NO:7.

3. The homodimeric protein according to any one of claim 1 or 2, said targeting unit comprising an amino acid sequence having at least 80 % sequence identity to the amino acid sequence of SEQ ID NO:9.

4. The homodimeric protein according to any one of claims 1-3, said targeting unit comprising an amino acid sequence having at least 80 % sequence identity to the amino acid sequence of SEQ ID NO:11.

5. The homodimeric protein according to any one of claims 1-4, wherein said antigenic unit is any protein unit such as a domain, short segment or a peptide or combinations of different protein units, derived from a pathogen or cancer related tissue.

6. The homodimeric protein according to any one of claims 1-5, wherein said targeting unit, dimerization motif and antigenic unit in said amino acid chain are in the N-terminal to C-terminal order of targeting unit, dimerization motif and antigenic unit.

7. The homodimeric protein according to any one of claims 1-6, wherein said targeting unit comprises an amino acid sequence having at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity to the amino acid sequence of SEQ ID NO:7.

8. The homodimeric protein according to any one of claims 1-7, wherein said targeting unit comprises an amino acid sequence having at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at

least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity to the amino acid sequence of SEQ ID NO:9.

9. The homodimeric protein according to any one of claims 1-8, wherein said targeting unit consists of an amino acid sequence having at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity to the amino acid sequence of SEQ ID NO:11.

10. The homodimeric protein according to any one of claims 1-9, wherein the dimerization motif comprises a hinge region or a shortened CH2 domain and optionally another domain that facilitate dimerization, such as an immunoglobulin domain, optionally connected through a linker.

11. The homodimeric protein according to claim 10, wherein shortened CH2 domain is Ig derived, such as IgY, such as an Ig derived from avian, such as chicken.

12. The homodimeric protein according to any one of claims 10-11, wherein the hinge region has the ability to form one, two, or several covalent bonds.

13. The homodimeric protein according to claim 12, wherein the covalent bond is a disulphide bridge.

14. The homodimeric protein according to any one of claims 10-13, wherein the immunoglobulin domain of the dimerization motif is a carboxyterminal C domain, such as a constant CH3 or CH4 domain, or a sequence that is substantially identical to said C domain or a variant or a functional fragment thereof.

15. The homodimeric protein according to claim 14, wherein the carboxyterminal C domain is derived from IgY or IgG.

16. The homodimeric protein according to any one of claims 10-15, wherein the immunoglobulin domain of the dimerization motif has the ability to homodimerize.

17. The homodimeric protein according to any one of claims 10-16, wherein said immunoglobulin domain has the ability to homodimerize via noncovalent interactions.

18. The homodimeric protein according to claim 17, wherein said noncovalent interactions are hydrophobic interactions.

19. The homodimeric protein according to any one of claims 1-18, wherein said dimerization domain comprise a domain selected from CH2, CH3, and CH4 or functional fragment thereof, such as a CH2 and/or a CH4 domain or functional fragment thereof from IgY.

20. The homodimeric protein according to any one of claims 1-19, wherein the dimerization motif consist of hinge exons h1 and h4 connected through a linker to a C_H3 domain of human or mouse, IgG, such as IgG3 or to C_H4 domain of avian IgY.

21. The homodimeric protein according to any one of claims 1-20, wherein the dimerization motif consist of an amino acid sequence having at least 80 % sequence identity to the amino acid sequence of SEQ ID NO:13.

22. The homodimeric protein according to any one of claims 10-21, wherein said linker is a $G_3S_2G_3SG$ linker.

23. The homodimeric protein according to any one of claims 1-22, wherein said antigenic unit and the dimerization motif is connected through a linker, such as a GLGGL linker or a GLSGL linker.

24. The homodimeric protein according to any one of claims 1-23, wherein said targeting unit consists of SEQ ID NO:11, or a variant thereof.

25. The homodimeric protein according to any one of claims 1-24, which homodimeric protein binds specifically to chicken MHC class II molecule on avian cells with a K_d lower than 0,1 nM, such as lower than 50 pM, such as lower than 40, 30, 20 or 10 pM.

26. The homodimeric protein according to any one of claims 1-25, wherein said antigenic unit comprises an antigenic unit suitable for preventive and/or therapeutic effect for poultry diseases, such as an antigen selected from agents causing avian coccidiosis, avian encephalomyelitis, avian infectious bronchitis, such as S protein, S1 protein or S2 protein, avian infectious bursal disease, such as VP2 protein, avian reovirus, such as Sigma Cc protein, chicken anaemia virus, duck virus enteritis, egg drop syndrome 1976, erysipelas, infectious laryngotracheitis, Marek's disease, Newcastle disease, such as Hemagglutinin-neuraminidase fusion protein, pasteurellosis, post-natal colibacillosis, salmonellosis, swollen

head syndrome, turkey haemorrhagic enteritis, turkey rhinotracheitis and avian influenza, such as HA proteins, such as HA1, HA2, HA3, HA4, HA5, HA6, HA7, HA8, HA9, HA10, HA11, HA12, HA13, HA14, or HA15.

27. The homodimeric protein according to any one of claims 1-26, wherein said antigenic unit comprises an amino acid sequence having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity to any one amino acid sequence selected from SEQ ID NO:23, SEQ ID NO:30, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, or SEQ ID NO:40, or a fragment thereof.

28. The homodimeric protein according to any one of claims 1-27, wherein said antigenic unit consists of an amino acid sequence having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity to any one amino acid sequence selected from SEQ ID NO:23 or SEQ ID NO:30, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, or SEQ ID NO:40, or a fragment thereof.

29. The homodimeric protein according to any one of claims 1-28, in its mature form without any signal peptide sequence.

30. The homodimeric protein according to any one of claims 1-29, wherein said avian are poultry, such as any one selected from Chicken, Duck, Emu, Goose, Indian Peafowl, Mute Swan, Ostrich, Pigeon, Turkey, Guineafowl, Common Pheasant, Golden Pheasant, and Rhea.

31. The homodimeric protein according to claim 30, wherein said avian is Chicken.

32. The homodimeric protein according to any one of claims 1-31, comprising an amino acid sequence having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at

least 99% sequence identity to any one amino acid sequence selected from the hCH3 IgG3 domain of SEQ ID NO:17, the hinge region of SEQ ID NO:14, the hinge region of SEQ ID NO:15, the linker of SEQ ID NO:16, SEQ ID NO:18, or SEQ ID NO:19, the hinge region of SEQ ID NO:20 or SEQ ID NO:21, the CH3 hIgG3 domain of SEQ ID NO:22, the construct of SEQ ID NO:24, SEQ ID NO:31, or SEQ ID NO:32, the Chicken IgY CH2 domain of SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, or SEQ ID NO:29, the Chicken IgY CH4 of SEQ ID NO:26, or any functional fragment thereof.

33. An amino acid chain comprising (1) an optional signal peptide, (2) a targeting unit, (3) an optional dimerization motif, and (4) an antigenic unit, said targeting unit being a scFv fragment specifically binding chicken MHC class II molecule on avian cells.

34. The amino acid chain according to claim 33, comprising a dimerization motif, and being able to form a homodimeric protein according to any one of clams 1-32.

35. A nucleic acid molecule, such as a DNA, encoding the amino acid chain according to any one of claims 33-34.

36. The nucleic acid molecule according to claim 35, which nucleic acid molecule is avian codon optimized.

37. A nucleic acid molecule comprising or consisting of any one of nucleotide sequences selected from the list consisting of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, nucleotides 1-36 of SEQ ID NO:12, nucleotides 37-81 of SEQ ID NO:12, nucleotides 82-111 of SEQ ID NO:12, nucleotides 112-432 of SEQ ID NO:12, nucleotides 433-447 of SEQ ID NO:12, or a variant thereof.

38. A vector, such as a viral vector or a plasmid vector, such as one compatible for avians comprising the nucleic acid molecule according to claims 35-37.

39. The vector according to claim 38 being able to express the nucleic acid molecule as a functional protein in avian cells

40. The nucleic acid molecule according to any one of claims 35-37 or vector of claims 38-39 formulated for administration to the subject, such as an avian population to induce production of the homodimeric protein in said subject.

41. The homodimeric protein according to any one of claims 1-32, or an amino acid chain according to claims 33-34, or the nucleic acid molecule according to any one of claims 35-37, or vector of claims 38-39 for use as a medicament.

42. A pharmaceutical composition comprising the homodimeric protein according to any one of claims 1-32, or an amino acid chain according to claims 33-34, or the nucleic acid molecule according to any one of claims 35-37, or vector of claims 38-39..

43. A host cell comprising the nucleic acid molecule according to any one of claims 35-37, or the vector according to claims 38-39.

44. A method for preparing a homodimeric protein according to any one of claims 1-32, or an amino acid chain of claims 33-34, the method comprising

a) transfecting the nucleic acid molecule according to any one of claims 35-37, or the vector according to claims 38-39 into a cell population;

b) culturing the cell population;

c) collecting and purifying the homodimeric protein, or amino acid chain expressed from the cell population.

45. A method for preparing a vaccine, such as a DNA vaccine, comprising an immunologically effective amount of a nucleic acid molecule according to any one of claims 35-37, or the vector according to claims 38-39 the method comprising

a. preparing a nucleic acid molecule according to any one of claims 35-37 or the vector according to claims 38-39; and

b. dissolving the nucleic acid molecule or vector obtained under step a) in a pharmaceutically acceptable carrier, diluent, or buffer.

46. A method for preparing a cell, such as an antigen presenting cell vaccine or cell line producing the homodimeric protein according to any one of claims 1-32, or the amino acid chain according to claims 33-34, the method comprising

a. preparing a nucleic acid molecule according to any one of claims 35-37, or vector of claims 38-39;

b. activating *in vitro* the cells, such as antigen presenting cells with an immunologically effective amount of a nucleic acid molecule or vector prepared under step a); and

c. preparing the cells, such as antigen presenting cells obtained under step b) in a suitable diluent, such as a pharmaceutically acceptable carrier, diluent, or buffer.

47. A vaccine against a disease or condition in an animal, such as a cancer or an infectious disease caused by a virus, bacteria or other infectious agent, the vaccine comprising an immunologically effective amount of a homodimeric protein according to any one of claims 1-32, or an amino acid chain according to claims 33-34, or nucleic acid molecule, such as a DNA, according to any one of claims 35-37, or vector of claims 38-39 wherein said vaccine is able to trigger both a T-cell- and B-cell immune response.

48. The vaccine according to claim 47 further comprising a pharmaceutically acceptable carrier and/or adjuvant.

49. A method of treating or preventing a disease or condition in an animal, such as a cancer or an infectious disease caused by a virus, bacteria or other infectious agent, the method comprising administering to said animal in need thereof, a homodimeric protein according to any one of claims 1-32, or an amino acid chain according to claims 33-34, or the nucleic acid molecule, such as a DNA, according to one of claims 35-37 or vector of claims 38-39.

50. The method according to claim 49, wherein the method comprises administering to the subject, such as an avian population in need thereof of a nucleic acid molecule, such as a DNA, according to one of claims 35-37 or vector of claims 38-39 with a subsequent step of electroporation.

51. The method according to claims 49 or 50, wherein the administration is performed intra-dermal or intra-muscular, respiratorial, mucosal, via the GI tract, or in ovo.

Figure 1

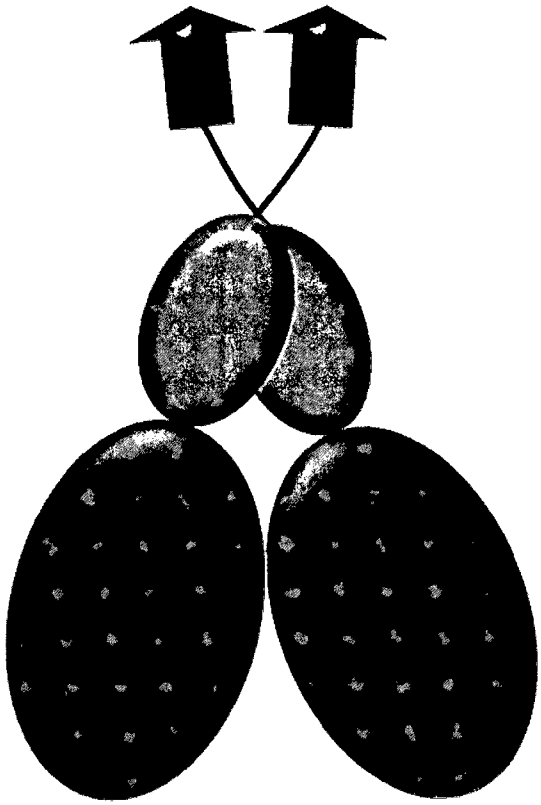


Figure 2

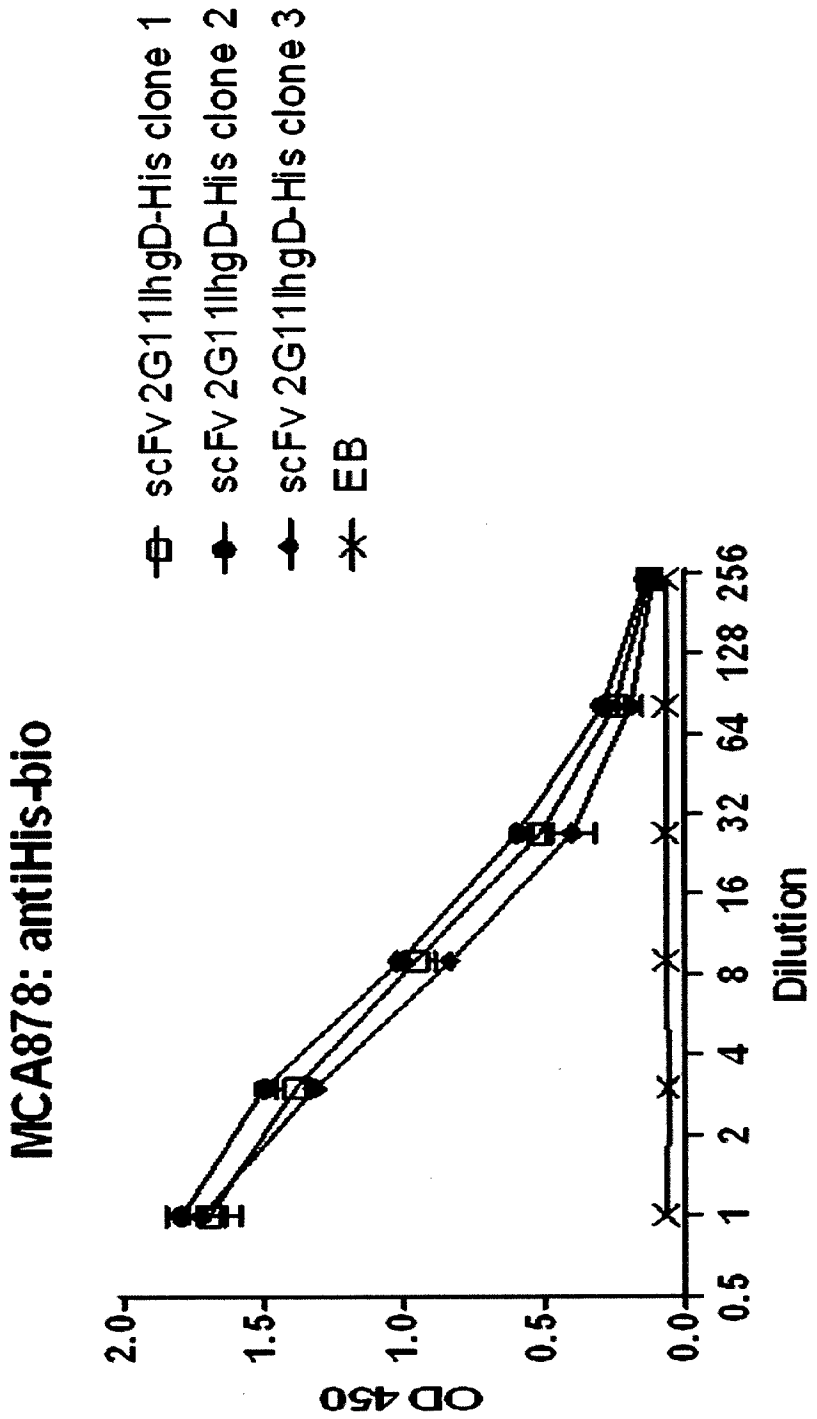

scFv 2G11lhgD-His clone 1
scFv 2G11lhgD-His clone 2
scFv 2G11lhgD-His clone 3
EB
MCA878: antiHis-bio
OD 450
2.0
1.5
1.0
0.5
0.0
0.5
1
2
4
8
16
32
64
128
256
Dilution

Figure 3

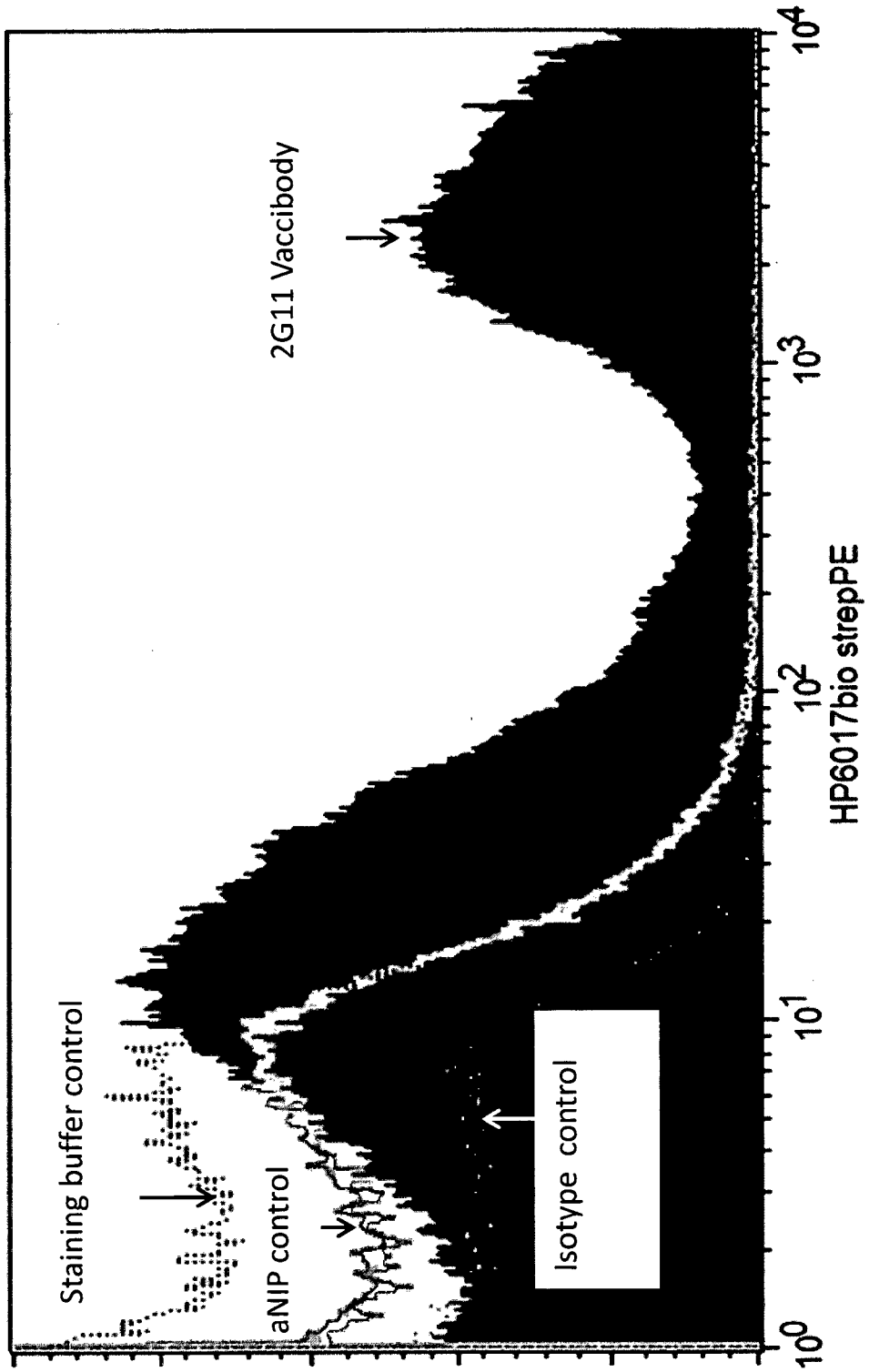

Staining buffer control
aNIP control
2G11 Vaccibody
Isotype control
HP6017bio strepPE
10^0
10^1
10^2
10^3
10^4

Figure 4

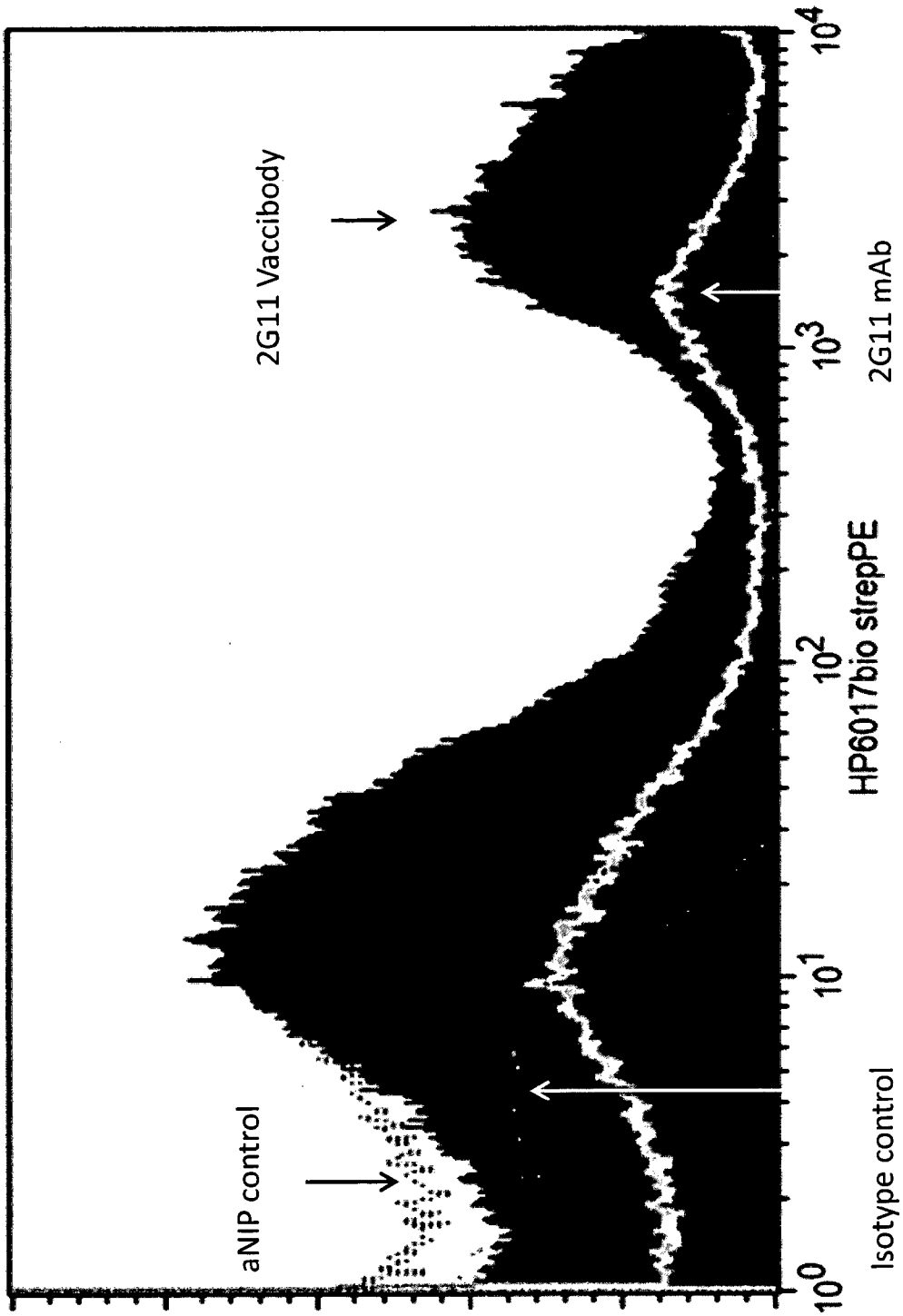
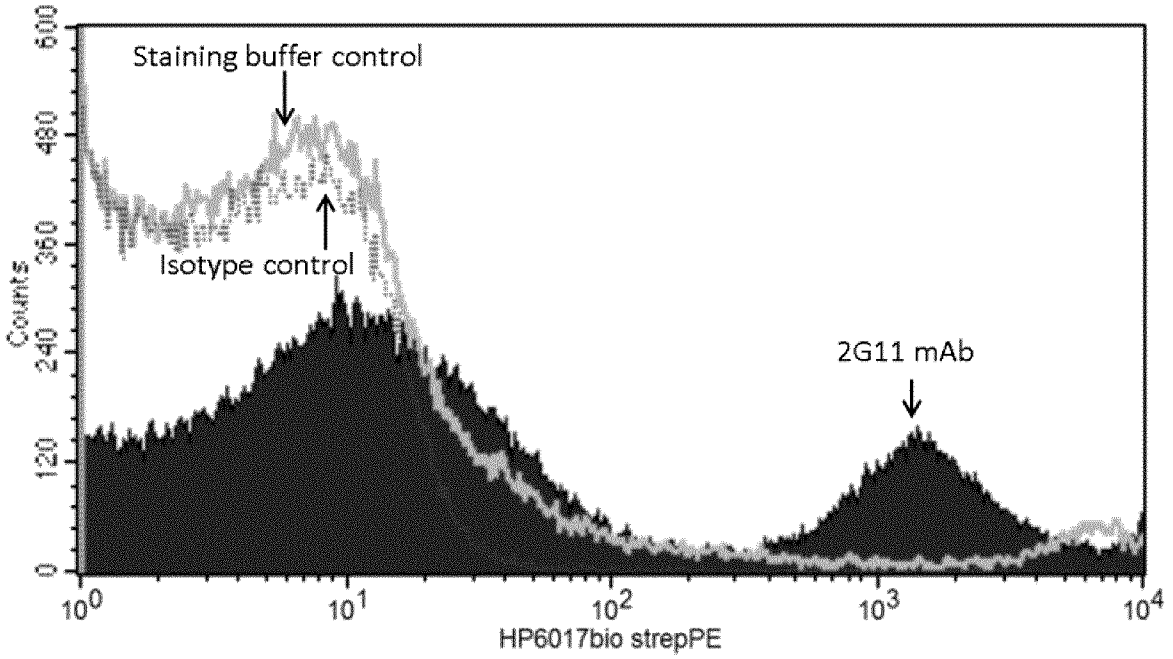

2G11 Vaccibody
aNIP control
Isotype control
2G11 mAb
HP6017bio strepPE
10^0
10^1
10^2
10^3
10^4

Figure 5


Staining buffer control
Isotype control
2G11 mAb
Counts
0
120
240
360
480
600
10^0
10^1
10^2
10^3
10^4
HP6017bio strepPE

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/054961

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61K39/395
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2007/297977 A1 (YU GUO-LIANG [US] ET AL) 27 December 2007 (2007-12-27) page 21 - page 22; sequence 18	37,38,43
X	WO 03/104428 A2 (VACCINEX INC [US]; UNIV ROCHESTER [US]) 18 December 2003 (2003-12-18) sequence 151	37
X	EP 2 103 628 A1 (FORERUNNER PHARMA RES CO LTD [JP]) 23 September 2009 (2009-09-23) sequence 91	37
X	WO 2011/124370 A1 (CORIMMUN GMBH [DE]; GAWAZ MEINRAD [DE]; SCHOENBERGER TANJA [DE]; DEGEN) 13 October 2011 (2011-10-13) sequence 5	37

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 May 2014	05/06/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Pérez-Mato, Isabel

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2004/076489 A1 (MEDINNOVA AS [NO]; BOGEN BJARNE [NO]; FREDRIKSEN AGNETE BRUNSVIK [NO];) 10 September 2004 (2004-09-10) the whole document	1-36, 39-42, 44-51
Y	MARIANNE FRØYLAND ET AL: "Targeted idiotype-fusion DNA vaccines for human multiple myeloma: preclinical testing", EUROPEAN JOURNAL OF HAEMATOLOGY, vol. 86, no. 5, 1 May 2011 (2011-05-01), pages 385-395, XP055056142, ISSN: 0902-4441, DOI: 10.1111/j.1600-0609.2011.01590.x the whole document	1-36, 39-42, 44-51
Y	Ã YNEBRÃTEN I ET AL: "P19-39. Vaccibodies: a novel vaccine strategy for HIV that target viral antigens to APC", RETROVIROLOGY, BIOMED CENTRAL LTD., LONDON, GB, vol. 6, no. Suppl 3, 22 October 2009 (2009-10-22), page P359, XP021064082, ISSN: 1742-4690, DOI: 10.1186/1742-4690-6-S3-P359 the whole document	1-36, 39-42, 44-51
Y	ÖYNEBRÅTEN I ET AL: "Generation of Antibody-Producing Hybridomas Following One Single Immunization with a Targeted DNA Vaccine", SCANDINAVIAN JOURNAL OF IMMUNOLOGY, BLACKWELL SCIENCE PUBL., OXFORD, GB, vol. 75, no. 4, 1 April 2012 (2012-04-01), pages 379-388, XP002690477, ISSN: 0300-9475, DOI: 10.1111/J.1365-3083.2011.02639.X the whole document	1-36, 39-42, 44-51
Y	FREDRIKSEN ET AL: "DNA Vaccines Increase Immunogenicity of Idiotypic Tumor Antigen by Targeting Novel Fusion Proteins to Antigen-Presenting Cells", MOLECULAR THERAPY, NATURE PUBLISHING GROUP, GB, vol. 13, no. 4, 1 April 2006 (2006-04-01), pages 776-785, XP005358612, ISSN: 1525-0016, DOI: 10.1016/J.YMTHE.2005.10.019 the whole document	1-36, 39-42, 44-51

-/--

2

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SWAYNE ET AL: "Avian influenza vaccines and therapies for poultry", COMPARATIVE IMMUNOLOGY, MICROBIOLOGY AND INFECTIOUS DISEASES, PERGAMON PRESS, OXFORD, GB, vol. 32, no. 4, 1 July 2009 (2009-07-01), pages 351-363, XP026029217, ISSN: 0147-9571, DOI: 10.1016/J.CIMID.2008.01.006 [retrieved on 2008-04-28] the whole document -----	1-36, 39-42, 44-51
Y	SHIRLEY M W ET AL: "Live attenuated vaccines against avian coccidiosis: Success with precocious and egg-adapted lines of Eimeria", PARASITOLOGY TODAY, ELSEVIER CURRENT TRENDS, vol. 13, no. 12, 1 December 1997 (1997-12-01), pages 481-484, XP002321768, ISSN: 0169-4758, DOI: 10.1016/S0169-4758(97)01153-8 the whole document -----	1-36, 39-42, 44-51
Y	VEITS JUTTA ET AL: "Newcastle disease virus expressing H5 hemagglutinin gene protects chickens against Newcastle disease and avian influenza", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, NATIONAL ACADEMY OF SCIENCES, US, vol. 103, no. 21, 23 May 2006 (2006-05-23) , pages 8197-8202, XP002415859, ISSN: 0027-8424, DOI: 10.1073/PNAS.0602461103 the whole document -----	1-36, 39-42, 44-51
Y	GOSSE BIJLENGA ET AL: "Development and use of the H strain of avian infectious bronchitis virus from the Netherlands as a vaccine: a review", AVIAN PATHOLOGY, vol. 33, no. 6, 1 December 2004 (2004-12-01), pages 550-557, XP055059674, ISSN: 0307-9457, DOI: 10.1080/03079450400013154 the whole document -----	1-36, 39-42, 44-51

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NIERI P ET AL: "Antibodies for Therapeutic Uses and the Evolution of Biotechniques", CURRENT MEDICINAL CHEMISTRY, BENTHAM SCIENCE PUBLISHERS, NL, vol. 16, no. 6, 1 February 2009 (2009-02-01), pages 753-779, XP008113274, ISSN: 0929-8673, DOI: 10.2174/092986709787458380 the whole document in particular, figure 6 and page 766	1-51
Y,P	WO 2013/092875 A1 (VACCIBODY AS [NO]) 27 June 2013 (2013-06-27) the whole document	1-36, 39-42, 44-51
Y,P	GUNNVEIG GRØDELAND ET AL: "The Specificity of Targeted Vaccines for APC Surface Molecules Influences the Immune Response Phenotype", PLOS ONE, vol. 8, no. 11, 11 November 2013 (2013-11-11), pages e80008-e80008, XP055120519, ISSN: 1932-6203, DOI: 10.1371/journal.pone.0080008 the whole document	1-36, 39-42, 44-51
Y,P	G. GRODELAND ET AL: "DNA Vaccine that Targets Hemagglutinin to MHC Class II Molecules Rapidly Induces Antibody-Mediated Protection against Influenza", THE JOURNAL OF IMMUNOLOGY, vol. 191, no. 6, 15 September 2013 (2013-09-15), pages 3221-3231, XP055120524, ISSN: 0022-1767, DOI: 10.4049/jimmunol.1300504 the whole document	1-36, 39-42, 44-51

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2014/054961

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2007297977 A1	27-12-2007	US 2007297977 A1	27-12-2007
		US 2011003399 A1	06-01-2011
WO 03104428 A2	18-12-2003	AU 2003274463 A1	22-12-2003
		AU 2009217460 A1	15-10-2009
		CA 2488682 A1	18-12-2003
		CN 1684700 A	19-10-2005
		EP 1531848 A2	25-05-2005
		EP 2070949 A2	17-06-2009
		JP 4643255 B2	02-03-2011
		JP 2005528914 A	29-09-2005
		NZ 537579 A	27-10-2006
		US 2004063907 A1	01-04-2004
		US 2008089886 A1	17-04-2008
		US 2009297440 A1	03-12-2009
		US 2009305350 A1	10-12-2009
		WO 03104428 A2	18-12-2003
EP 2103628 A1	23-09-2009	AU 2007332473 A1	19-06-2008
		CA 2672581 A1	19-06-2008
		CN 101663322 A	03-03-2010
		EP 2103628 A1	23-09-2009
		US 2010111852 A1	06-05-2010
		WO 2008072723 A1	19-06-2008
WO 2011124370 A1	13-10-2011	AU 2011238084 A1	01-11-2012
		CA 2795142 A1	13-10-2011
		CN 102906116 A	30-01-2013
		EA 201201234 A1	30-04-2013
		EP 2377888 A1	19-10-2011
		EP 2556092 A1	13-02-2013
		JP 2013532954 A	22-08-2013
		US 2013130315 A1	23-05-2013
		WO 2011124370 A1	13-10-2011
WO 2004076489 A1	10-09-2004	AU 2004215489 A1	10-09-2004
		CA 2517054 A1	10-09-2004
		EP 1599504 A1	30-11-2005
		US 2004253238 A1	16-12-2004
		US 2012171242 A1	05-07-2012
		WO 2004076489 A1	10-09-2004
WO 2013092875 A1	27-06-2013	NONE	