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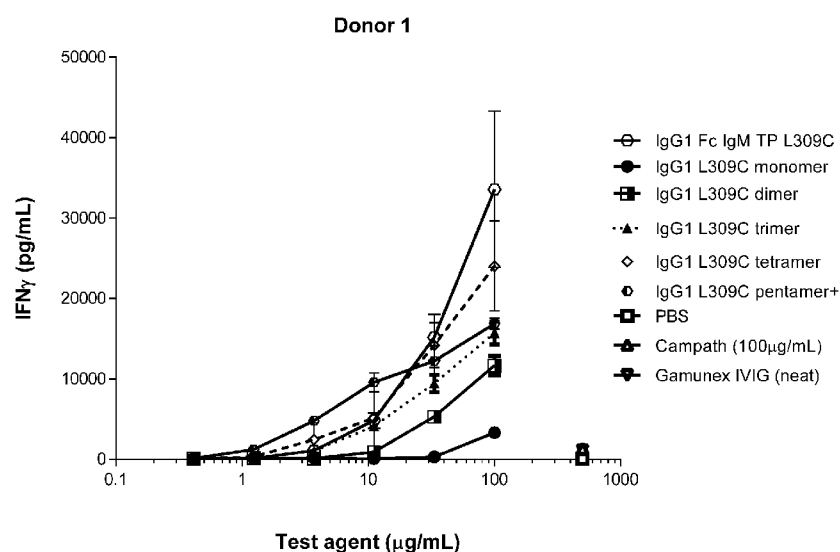
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(54) **Title:** MULTIMERIC FC PROTEINS

Figure 2

(57) **Abstract:** The invention relates to multimeric proteins which bind to human Fc-receptors. The invention also relates to therapeutic compositions comprising the multimeric proteins, and their use in the treatment of cancer.

MULTIMERIC FC PROTEINS

The invention relates to multimeric proteins which bind to human Fc-receptors. The invention also relates to therapeutic compositions comprising the multimeric proteins, and their use in the treatment of cancer.

BACKGROUND

Monoclonal antibodies (Mabs) are widely used in the treatment of cancer. They benefit from favourable pharmacokinetics and target specificity but are often lacking in tumour penetration and/or accumulation and in potency of cell killing via antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) pathways. Efficacy often combines a blend of direct action on the target (blockade, internalisation) and target killing through ADCC. CDC has rarely been observed to be a major mechanism of killing. Potency of Mabs has been increased by conjugation or fusion to drugs, or by Fc-engineering or glycan-engineering (bisecting GlcNc, afucosylated) to improve their affinity for FcγRIIIa. FcγRIIIa is predominantly found in natural killer (NK) cells, hence engineering improvements have largely been focussed on improving the effective involvement of NK cells. Bispecific antibodies have focussed on the recruitment of T-cells to tumours. Recently attempts have been made to improve CDC through 'on tumour' hexamerisation of Mabs (Diebold, Science 2014).

Cytokines such as IL-2 (for example Proleukin®), TNFα (for example Beromun®) and IFNγ (for example Actimmune®) have been trialled in or approved for clinical use in oncology and other fields. These very potent immune-modulators are typically restricted in their use by dose limiting toxicity after systemic administration.

There remains a significant clinical need to for effective and potency of biological products for the treatment of cancer.

Multimeric proteins comprising antibody Fc-domains are known in the art. (Mekhaie et al; Nature Scientific Reports 1:124, published 19th October 2011).

The present invention is based on the discovery that certain forms of these and related proteins, and constructs comprising those proteins, cause the release of at least of the following: IFNγ and TNFα and release minor amounts of IL-6 and Il-8. We disclose their use to develop new improved therapies for the treatment of cancer.

SUMMARY OF THE DISCLOSURE

The present invention provides a multimeric protein comprising two or more polypeptide monomer units; wherein each polypeptide monomer unit comprises two heavy chain Fc-regions; said multimeric protein being capable of modulating the release of cytokines in a cytokine release assay.

Suitable cytokine release assays for testing and identifying multimeric proteins of the invention are known in the art. An example of a suitable cytokine release assay is provided herein and in Example 1.

In one example, each heavy chain Fc-region comprises one or more mutations that modulate cytokine release compared to the same multimeric protein without such mutations. In one embodiment, the multimeric protein of the invention increases cytokine release. In one embodiment, the multimeric protein of the invention decreases cytokine release.

In one embodiment the mutations increase cytokine release, for example increase one or more cytokines independently selected from the group comprising IFN γ , TNF α , IL-6 and IL-8.

In one example one or more mutations to increase cytokine release are independently selected from the group comprising K322A, P331A and S298A.

In one example one or more mutations to increase cytokine release are selected from the group consisting of:

- leucine residue at position 234
- alanine residue at position 327
- tyrosine residue at position 296

In one embodiment the mutations decrease cytokine release, for example decrease one or more cytokines independently selected from the group comprising IFN γ , TNF α , IL-6 and IL-8.

In one example one or more mutations to decrease cytokine release are independently selected from the group comprising L234A, L235A, S267E, L328F, 236R, and L328R.

In one example one or more mutations to decrease cytokine release are selected from the group consisting of:

- phenylalanine residue at position 234
- glycine residue at position 327
- phenylalanine residue at position 296

In one embodiment, each heavy chain Fc-region is fused at its C-terminal to a tailpiece which causes the monomer units to assemble into a multimer.

- 5 In one embodiment, the heavy chain Fc-region comprises a histidine residue at position 310 and any amino acid residue other than cysteine at position 309. In one embodiment, the heavy chain Fc-region comprises any amino acid residue other than cysteine at position 309 and any amino acid residue other than histidine at position 310. In one embodiment, the heavy chain Fc-region comprises a cysteine residue at position 309 and a leucine residue at
10 position 310. In one embodiment, the heavy chain Fc-region comprises a cysteine residue at position 309 and a histidine residue at position 310. In one embodiment, each heavy chain Fc-region comprises a cysteine residue at position 309 which causes the monomer units to assemble into a multimer.
- 15 In one embodiment, the multimeric protein of the invention binds to human FcRn.

The present invention also provides a method for measuring cytokine release by a multimeric protein of the invention, comprising:

- (a) adding the multimeric protein to a sample of whole blood;
20 (b) incubating the sample at 37°C without CO₂ supplementation for 24 hours;
(c) removing the serum;
(d) analysing the serum for the presence of cytokines.

DETAILED DESCRIPTION OF THE DISCLOSURE

- 25 Multimeric protein as employed herein refers to a protein comprising two, three, four, five or six monomer units assembled to behave as single molecule, for example an Fc-multimer.

- Polypeptide monomer units (also referred to as polypeptide units) as employed herein refer to the repeating units, which are assembled to provide the multimeric proteins of the present
30 disclosure. Having said that a multimeric proteins of the present disclosure may be prepared from one or two types of units, for example combined in a specific ratio, for example 1:5, 2:4, 3:3, 4:2 or 5:1.

- The polymeric monomer unit comprises Fc-heavy chains (referred to herein as Fc-regions).
35 Thus the monomer unit is polypeptide dimer of Fc-heavy chains. In one embodiment the monomer unit comprises a homodimer of heavy chains. That is to say both heavy chains making up the unit are the same or identical. In one embodiment the monomer unit

comprises a heterodimer of heavy chains. That is to the heavy chains making up the dimer are different.

Examples of homodimers, include the use of knobs and holes technology. 'Knobs-into-holes' was originally proposed by Crick in 1952 as a model for the packing of amino acid side chains between adjacent α -helices. 'Knobs-and-holes' is an effective design strategy for engineering antibody heavy chain homodimers for heterodimerization. In one embodiment a 'knob' variant is first obtained by replacement of a small amino acid with a larger one in a constant domain of a heavy chains, for example a CH2 or CH3 domain, such as T366Y.

The knob is designed to insert into a 'hole' in the corresponding heavy chain domain, for example CH2 or CH3 domain created by judicious replacement of a large residue with a smaller one, such as Y407T.

Alternative arrangement for knob-and-holes can routinely be prepared by persons skilled in the art.

The dimer of the Fc-heavy chains is referred to herein as the Fc-domain in the multimers of the present disclosure.

In one embodiment, one or each heavy chain Fc-region is fused or linked, such as fused, to an antigen binding portion. In one embodiment, one of the heavy chain Fc-regions (Fc-chains) is fused to an antigen binding portion. In one embodiment, each of the heavy chain Fc-regions (Fc-chains) is fused to an antigen binding portion.

In one embodiment the antigen binding portion is a polypeptide sequence.

In one embodiment the antigen binding portion comprising 3 CDRs or more, for example wherein the CDRs are in an antibody variable domain or domains. In one embodiment the binding domain is selected from the group comprising V_{HH} , dAb, lipocalin and DARPin.

Linked as employed herein refers to wherein the binding domain is connected to the Fc-chain via a linker on conjugated thereto, for example an amino acid linker of 50 or less amino acids. In one embodiment the linker is based on repeating units of the sequence G4S.

Thus in one embodiment an antibody molecule, for example antibody or binding fragment is conjugated, for example using maleimide chemistry, to multimeric protein (for example which does not comprising a binding domain integral therewith).

An antibody molecule as employed herein includes a complete antibody molecule having full length heavy and light chains or a binding fragment thereof and may be, but are not limited to Fab, modified Fab, Fab', modified Fab', F(ab')₂, Fv, single domain antibodies (e.g. VH or VL or VHH), scFv, bi, tri or tetra-valent antibodies, Bis-scFv, diabodies, triabodies, tetrabodies and epitope-binding fragments of any of the above (see for example Holliger and Hudson, 2005, Nature Biotech. 23(9):1126-1136; Adair and Lawson, 2005, Drug Design Reviews - Online 2(3), 209-217). The methods for creating and manufacturing these antibody fragments are well known in the art (see for example Verma *et al.*, 1998, Journal of Immunological Methods, 216:165-181). Other antibody fragments for use in the present invention include the Fab and Fab' fragments described in International patent applications WO05/003169, WO05/003170 and WO05/003171. Multi-valent antibodies may comprise multiple specificities e.g. bispecific or may be monospecific (see for example WO92/22853, WO05/113605, WO2009/040562 and WO2010/035012).

Binding fragment of an antibody as employed herein refers to a fragment capable of binding an antigen with affinity to characterise the fragment as specific for the antigen.

Fused to as employed herein refers to the wherein the binding domain is directed connected to the Fc-chains, i.e. without a linker, for example connected by a peptide bond.

In one embodiment the binding domain is linked or fused to the N-terminus of a heavy chain (Fc-region). In one embodiment both heavy chains, in one or more polypeptide units, have a binding domain on the N-terminus. In one embodiment only one of the heavy chains, in one or more polypeptide units, have a binding domain on the N-terminus, for example one of the heavy chains in a heterodimer (such as knobs-and-holes) has a binding domain on the N-terminus.

In one embodiment the multimeric protein molecules according to the present disclosure have one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve binding domains, for example one to six binding domains.

In one embodiment the multimeric protein according to the present disclosure does not comprise a binding domain (but for example may be conjugate to one).

In one embodiment the binding domain or domains has/have low affinity for the antigen/target to which it/they are specific. Low affinity as employed herein refers to 500nM to 100pM, such as 100nM to 500pM, in particular 10nM to 1nM. Affinity can be measured

by techniques known to those skilled in the art, such as surface plasmon resonance, in particular BIAcore.

In one embodiment the multimeric proteins according to the present disclosure have high avidity. Avidity can be measured using and ELISA titration, see for example Pericani et al Journal of Clinical Laboratory Analysis Volume 21, Issue 3, pages 201–206, 2007.

In one embodiment, the antigen binding portion binds to a cancer target. In embodiment the antigen is a tumor antigen (cancer antigen) that is an antigen specific to the tumor and not a self-antigen. In one embodiment the antigen is self-antigen.

The multimeric proteins that increase cytokine release may be particularly useful in breaking tolerance to self-antigens.

In one embodiment the antigen is a cancer testis antigen.

In one embodiment the antigen is an onco-fetal antigen.

Cancer targets include:

- a Mage gene product, for example MAGE tumour antigen, for example, MAGE 1, MAGE 2, MAGE 3, MAGE 4, MAGE 5, MAGE 6, MAGE 7, MAGE 8, MAGE 9, MAGE 10, MAGE 11 or MAGE 12. The genes encoding these MAGE antigens are located on chromosome X and share with each other 64 to 85% homology in their coding sequence (De Plaen, 1994). These antigens are sometimes known as MAGE A1, MAGE A2, MAGE A3, MAGE A4, MAGE A5, MAGE A6, MAGE A7, MAGE A8, MAGE A9, MAGE A 10, MAGE A1 1 and/or MAGE A12 (The MAGE A family). In one embodiment, the antigen is MAGE and/or an antigen from one of two further MAGE families may be used: the MAGE B and MAGE C group. The MAGE B family includes MAGE B 1 (also known as MAGE Xp 1 , and DAM 10), MAGE B2 (also known as MAGE Xp2 and DAM 6) MAGE B3 and MAGE B4 - the Mage C family currently includes MAGE C1 and MAGE C2;
- cancer testis antigens such as PRAME, LAGE 1, LAGE 2, and others, for example details of which can be obtained from www.cancerimmunity.org/CTdatabase;
- SSX-2; SSX-4; SSX-5; NA17; MELAN-A; Tyrosinase; LAGE-I; NY-ESO-I; PRAME; P790; P510; P835; B305D; B854; CASB618 (as described in WO00/53748); CASB7439 (as described in WO01/62778); C1491 ; C1584; and C1585. In one embodiment, the antigen may comprise or consist of P501S (also known as prostatein); and
- the antigen may comprise or consist of WT-I expressed by the Wilm's tumor gene, or its N-terminal fragment WT-IF comprising about or approximately amino acids 1-249;

- the antigen expressed by the Her-2/neu gene, or a fragment thereof.

The present invention also provides an isolated DNA sequence encoding a polypeptide chain of a polypeptide monomer unit of a multimeric protein according to the invention, or a component part thereof.

The present invention also provides a cloning or expression vector comprising one or more DNA sequences according to the invention.

The present invention also provides a host cell comprising one or more cloning or expression vectors according to the invention.

The present invention also provides a process for the production of a multimeric protein according to the invention, comprising culturing a host cell under conditions suitable for protein expression and assembly into multimers, and isolating and optionally purifying the multimeric protein.

The present invention also provides a pharmaceutical composition comprising a multimeric protein of the invention, in combination with a pharmaceutically acceptable excipient, diluent or carrier.

The present invention also provides the multimeric protein of the invention for use in therapy.

The present invention also provides the multimeric protein of the invention for use in the treatment of cancer.

The present invention also provides the use of the multimeric protein of the invention for the manufacture of a medicament for the treatment of cancer.

In one embodiment the cancer is a tumour. Tumour as employed herein is intended to refer to an abnormal mass of tissue that results from excessive cell division that is uncontrolled and progressive, also called a neoplasm. They may be either benign (not cancerous) or malignant. Tumour encompasses all forms of cancer and metastases.

In one embodiment the tumour is a solid tumour. The solid tumour may be localised or metastasised. In one embodiment the tumour is of epithelial origin. In one embodiment the tumour is a solid tumour.

In one embodiment the tumour is a malignancy, such as colorectal cancer, hepatoma (liver cancer), prostate cancer, pancreatic cancer, breast cancer, ovarian cancer, thyroid cancer, renal cancer, bladder cancer, head and neck cancer or lung cancer.

- 5 In one embodiment the tumour is a colorectal malignancy. Malignancy as employed herein means cancerous cells. In one embodiment the cancer is colorectal cancer and/or metastatic forms thereof such as liver metastasis.

10 In one embodiment the cancer is liver cancer and/or metastatic forms thereof. In one embodiment the cancer is lung cancer and/or metastatic forms thereof. In one embodiment the cancer is ovarian cancer and/or metastatic forms thereof, such as lung metastasis. In one embodiment the cancer is renal cancer and/or metastatic forms thereof. In one embodiment the cancer is bladder cancer and/or metastatic forms thereof. In one embodiment the cancer is throat cancer.

15 In one embodiment the cancer is skin cancer, such as melanoma. In one embodiment the cancer is Leukemia. In one embodiment the cancer is glioblastoma, medulloblastoma or neuroblastoma. In one embodiment the cancer is a neuroendocrine cancer. In one embodiment the cancer is Hodgkin's or non-Hodgkins lymphoma.

20 In one embodiment the oncolytic adenovirus is employed in the treatment or prevention of metastasis.

25 Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of skill in the art to which this invention belongs. All publications and patents referred to herein are incorporated by reference.

It will be appreciated that any of the embodiments described herein may be combined.

30 In the present specification the EU numbering system is used to refer to the residues in antibody domains, unless otherwise specified. This system was originally devised by Edelman et al, 1969 and is described in detail in Kabat et al, 1987.

Edelman *et al.*, 1969; "The covalent structure of an entire γ G immunoglobulin molecule," Biochemistry Vol.63 pp78-85.

35 Kabat *et al.*, 1987; in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA.

Where a position number and/or amino acid residue is given for a particular antibody isotype, it is intended to be applicable to the corresponding position and/or amino acid residue in any other antibody isotype, as is known by a person skilled in the art.

5 **FIGURES**

Figure 1: Example of (a) an expression construct and (b) a multimeric fusion protein according to the invention. SP is signal peptide, CH2 and CH3 are heavy chain constant domains, TP is tailpiece.

10 **Figure 2:** Stimulation of cytokine release by multimeric proteins without a tailpiece, assembled via L309C.

Figure 3: Stimulation of cytokine release by multimeric proteins comprising a tailpiece.

15 **Figure 4:** Example amino acid sequences of a polypeptide chain of a polypeptide monomer unit, for multimeric proteins comprising a tailpiece. In each sequence, the tailpiece sequence is underlined, and any mutations are shown in bold and underlined. The hinge is underlined and in italics. In constructs comprising a CH4 domain from IgM, this region is shown in italics.

20 **Figure 5:** Example amino acid sequences of a polypeptide chain of a polypeptide monomer unit, for multimeric proteins without a tailpiece. In each sequence, mutations are shown in bold and underlined. The optional hinge region is underlined. In constructs comprising a CH4 domain from IgM, this region is shown in italics.

EXAMPLES

Example 1: Human whole blood cytokine release assay

25 Fresh blood was collected from donors in lithium heparin vacutainers. The Fc-multimer constructs of interest or controls were serially diluted in sterile PBS to the indicated concentrations. 12.5µl of Fc-multimer or control was added to the assay plates, followed by 237.5µl of whole blood. The plate was incubated at 37°C without CO₂ supplementation for 24hrs. Plates were centrifuged at 1800rpm for 5 minutes and the serum removed for cytokine analysis. Cytokine analysis was performed by Meso Scale Discovery cytokine multiplex
30 according to the manufacturer's protocol and read on a Sector Imager 6000.

Results

Results for Fc multimers without a tailpiece, assembled via L309C, are shown in Figure 1. The data demonstrated a positive correlation between the number of monomer units in the multimeric protein and the level of cytokine released. Cytokine levels produced by tetramer

and higher order multimers were similar to those produced by purified hexameric IgG1 Fc/IgM tailpiece.

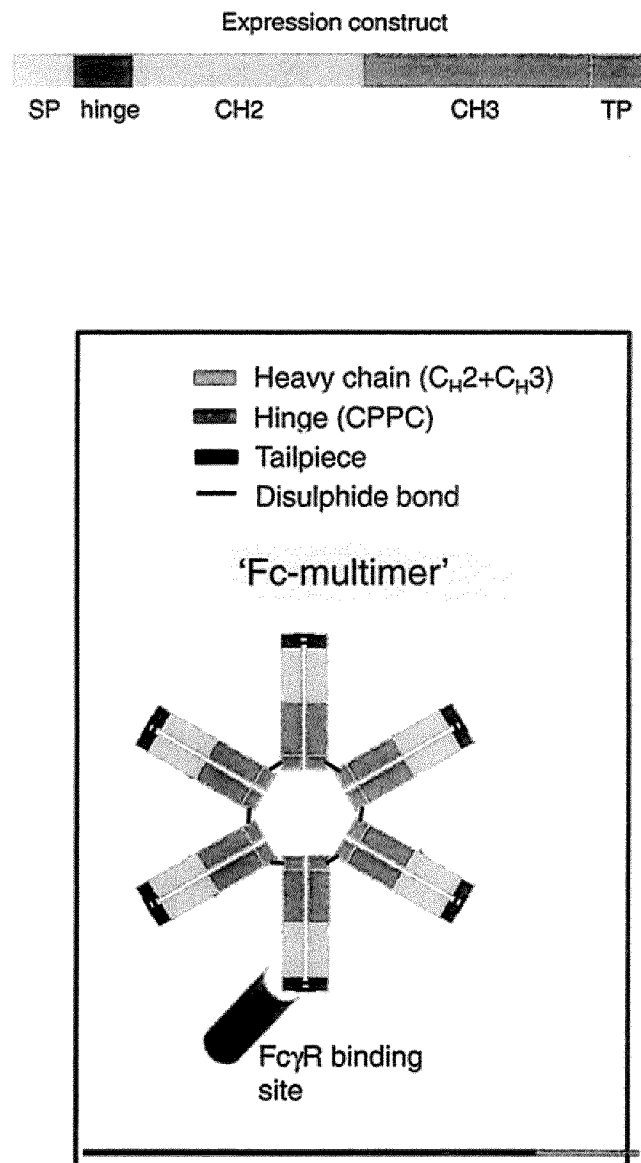
Results for Fc-multimers with a tailpiece are shown in Figure 2. The data demonstrated that IgG1 Fc-multimers, both with and without L309C, stimulate very high levels of cytokine release. The observed levels of cytokines were higher than those produced by the positive control, Campath. In marked contrast, IgG4 Fc-multimers, and IgG1 Fc-multimers comprising the FcγR and C1q inert “LALA” mutation (L234A L235A), produced virtually zero cytokine release.

CLAIMS

1. A multimeric protein comprising two or more polypeptide monomer units; wherein each polypeptide monomer unit comprises an antibody Fc-domain comprising two heavy chain Fc-regions; said multimeric protein being capable of stimulating the release of cytokines in a cytokine release assay.
2. The multimeric protein of claim 1, wherein each heavy chain Fc-region comprises one or more mutations that modulate cytokine release compared to the same multimeric protein without such mutations.
3. The multimeric protein of claim 1, wherein one or each heavy chain Fc-region is fused or linked to an antigen binding portion.
4. The multimeric protein of claims 1 to 3, wherein each heavy chain Fc-region is fused at its C-terminal to a tailpiece which causes the monomer units to assemble into a multimer.
5. The multimeric protein of claim 4, wherein the heavy chain Fc-region comprises a histidine residue at position 310 and any amino acid residue other than cysteine at position 309.
6. The multimeric protein of claim 4, wherein the heavy chain Fc-region comprises any amino acid residue other than cysteine at position 309 and any amino acid residue other than histidine at position 310.
7. The multimeric protein of claim 4, wherein the heavy chain Fc-region comprises a cysteine residue at position 309 and a leucine residue at position 310.
8. The multimeric protein of claim 4, wherein the heavy chain Fc-region comprises a cysteine residue at position 309 and a histidine residue at position 310.
9. The multimeric protein of claims 1 to 3, wherein each heavy chain Fc-region comprises a cysteine residue at position 309 which causes the monomer units to assemble into a multimer.

10. The multimeric protein of any of claims 1 to 9, wherein one of the heavy chain Fc-regions is fused to an antigen binding portion.
11. The multimeric protein of any of claims 1 to 9, wherein each of the heavy chain Fc-regions is fused to an antigen binding portion.
12. The multimeric protein of claim 10 or claim 11, wherein the antigen binding portion binds to a cancer target.
13. The multimeric protein of claim 10 or claim 11, wherein the antigen binding portion is selected from the group comprising vHH, dAb, lipocalin and DARPin.
14. The multimeric protein of claims 1 to 13, which increases cytokine release.
15. The multimeric protein of claims 1 to 13, which decreases cytokine release.
16. The multimeric protein of any preceding claim, which binds to human FcRn.
17. A method for measuring cytokine release by a multimeric protein of any of claims 1 to 16, comprising:
 - (a) adding the multimeric protein to a sample of whole blood;
 - (b) incubating the sample at 37°C without CO₂ supplementation for 24 hours;
 - (c) removing the serum;
 - (d) analysing the serum for the presence of cytokines.
18. An isolated DNA sequence encoding a polypeptide chain of a polypeptide monomer unit of a multimeric protein according to any of claims 1 to 16, or a component part thereof.
19. A cloning or expression vector comprising one or more DNA sequences according to claim 18.
20. A host cell comprising one or more cloning or expression vectors according to claim 19.

21. A process for the production of a multimeric protein according to any of claims 1 to 16, comprising culturing a host cell according to claim 20 under conditions suitable for protein expression and assembly into multimers, and isolating and optionally purifying the multimeric protein.
22. A pharmaceutical composition comprising a multimeric protein of any of claims 1 to 16, in combination with a pharmaceutically acceptable excipient, diluent or carrier.
23. The multimeric protein of any of claims 1 to 16 for use in therapy.
24. The multimeric protein of claim 23 for use in the treatment of cancer.
25. Use of the multimeric protein of any of claims 1 to 16 for the preparation of a medicament for the treatment of cancer.

Figure 1

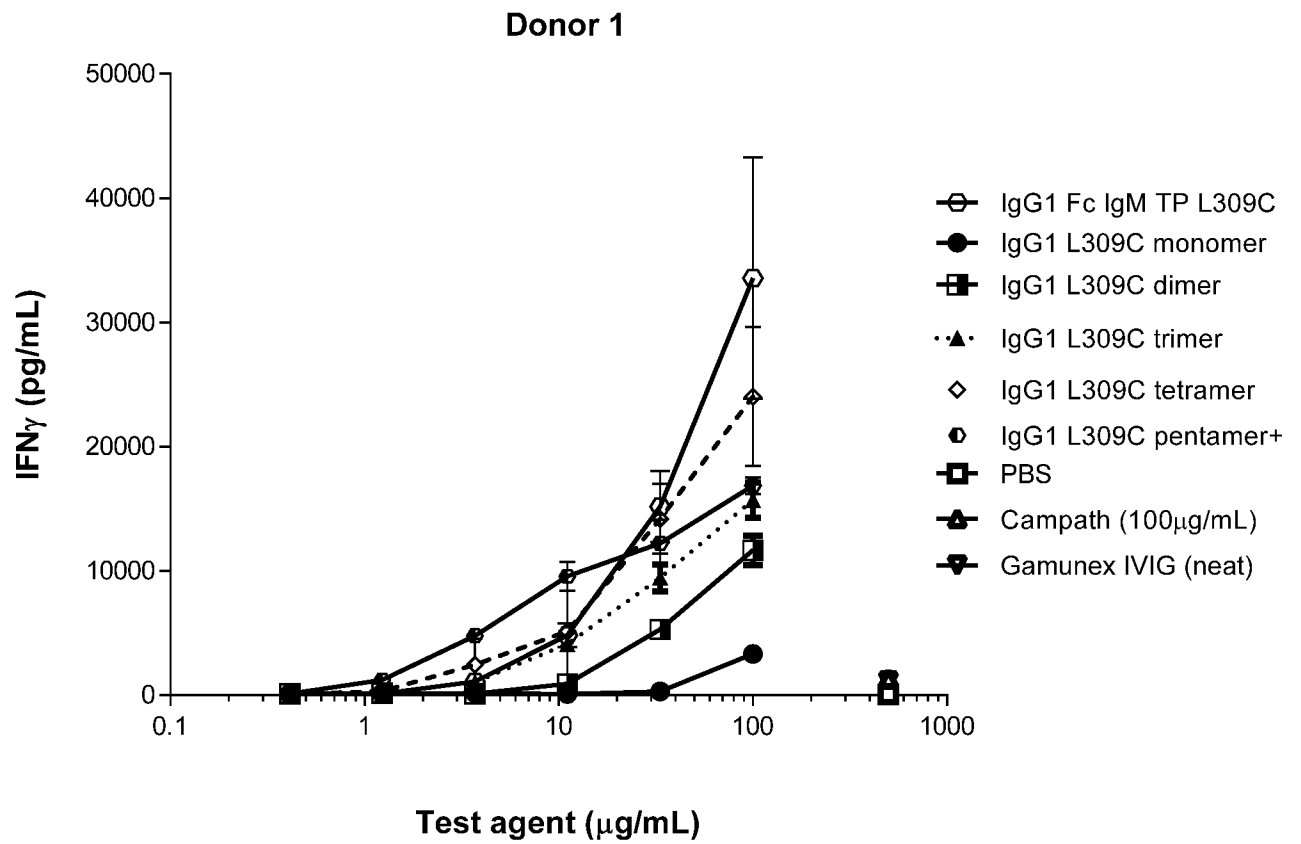


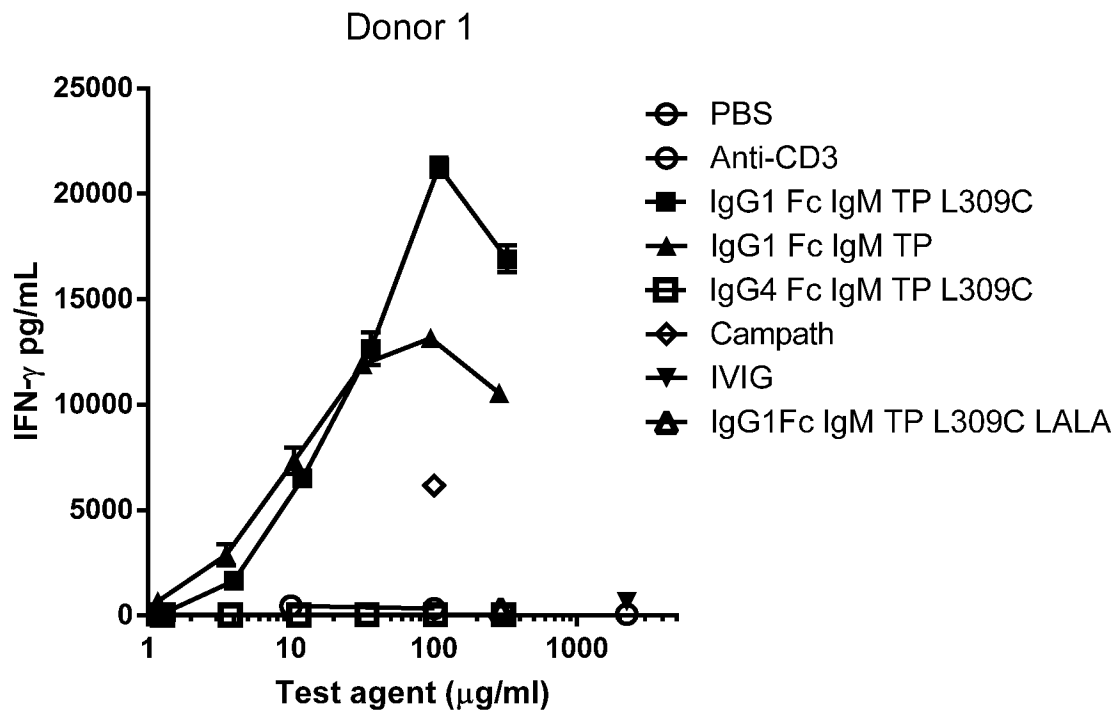
Figure 3

Figure 4**1. Human IgG1 Fc-multimer IgM tp SEQ ID NO:1**

CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
VDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSPGKPTLYNVSLVMSDTAGTCY

2. Human IgG4 Fc-multimer IgM tp SEQ ID NO:2

CPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA
KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVY
TLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLT
VDKSRWQEGNVVFSCSVMHEALHNHYTQKSLSLSLGKPTLYNVSLVMSDTAGTCY

3. Human IgG1 Fc-multimer IgA tp SEQ ID NO:3

CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
VDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSPGKPTHVNVSVVMAEVDGTCY

4. Human IgG4 Fc-multimer IgA tp SEQ ID NO:4

CPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA
KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVY
TLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLT
VDKSRWQEGNVVFSCSVMHEALHNHYTQKSLSLSLGKPTHVNVSVVMAEVDGTCY

5. Human IgG1 Fc-multimer Cμ4-IgM tp SEQ ID NO:5

CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
VDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSPGKVALHRPDVYLLPPAREQLNLRESATI
TCLVTGFSPADVVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETY
TCVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY

Figure 4 continued**6. Human IgG4 Fc-multimer C μ 4-IgM tp****SEQ ID NO:6**

CPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA
KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVY
TLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLT
VDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLKVALHRPDVYLLPPAREQLNLRESATI
TCLVTGFSPADV FVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETY
TCVAHEALPNRV TERTV DKSTGKPTLYNVSLVMSD TAGTCY

7. Human IgG1 Fc-multimer IgM tp S267A**SEQ ID NO:7**

CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV AHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
VDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGKPTLYNVSLVMSD TAGTCY

Figure 5**1. Human IgG1 Fc-multimer L309C SEQ ID NO:8**

CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVCHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLT
VDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK

2. Human IgG4 Fc-multimer L309C SEQ ID NO:9

CPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA
KTKPREEQFNSTYRVVSVLTVCHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVY
TLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLT
VDKSRWQEGNVFSQSVMEALHNHYTQKSLSLGLGK

3. Human IgG1 Fc-multimer L309C SEQ ID NO:10

CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVCHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLT
VDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK

4. Human IgG4 Fc-multimer L309C SEQ ID NO:11

CPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA
KTKPREEQFNSTYRVVSVLTVCHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVY
TLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLT
VDKSRWQEGNVFSQSVMEALHNHYTQKSLSLGLGK

5. Human IgG1 Fc-multimer C_μ4 L309C SEQ ID NO:12

CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVCHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLT
VDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGKVALHRPDVYLLPPAREQLNLRESATI
TCLVTGFSPADVVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETY
TCVAHEALPNRVTERTVDKSTGK

Figure 5 continued**6. Human IgG4 Fc-multimer C μ 4 L309C****SEQ ID NO:13**

CPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA
KTKPREEQFNSTYRVVSVLTVCHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVY
TLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSRLT
VDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKVALHRPDVYLLPPAREQLNLRESATI
TCLVTGFSPADVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETY
TCVAHEALPNRVTERTVDKSTGK

7. Human IgG1 Fc-multimer S267A L309C**SEQ ID NO:14**

CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVAHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVCHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLT
VDKSRWQQGNVFNCSVMHEALHNHYTQKSLSLSPGK

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/067404

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/00 A61P35/00
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

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

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Further documents are listed in the continuation of Box C.



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* Special categories of cited documents :

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2016/067404

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International application No

PCT/EP2016/067404

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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International application No

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