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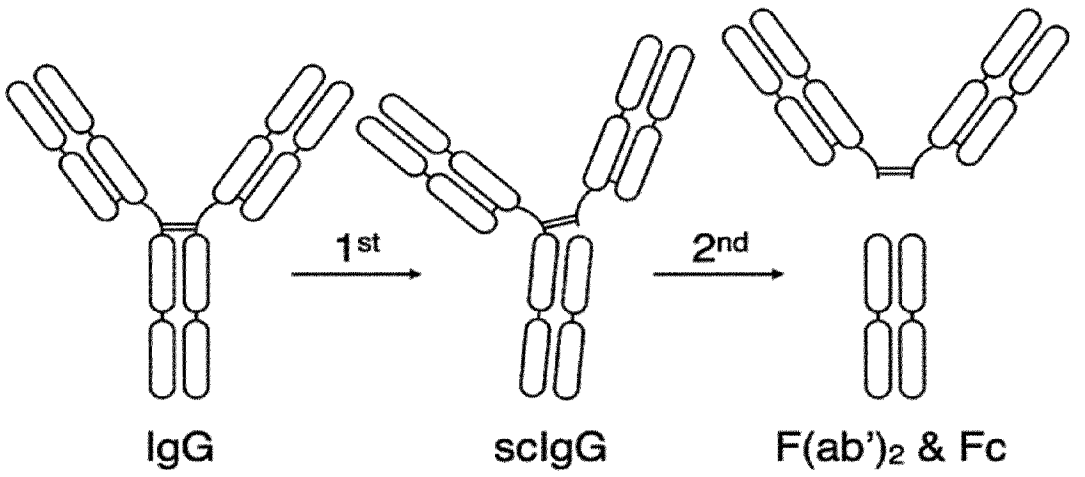

(57) **Abrégé/Abstract:**
The present invention relates to a novel polypeptide which displays IgG cysteine protease activity, and in vivo and ex vivo uses thereof. Uses of the polypeptide include methods for the prevention or treatment of diseases and conditions mediated by IgG, and methods for the analysis of IgG.

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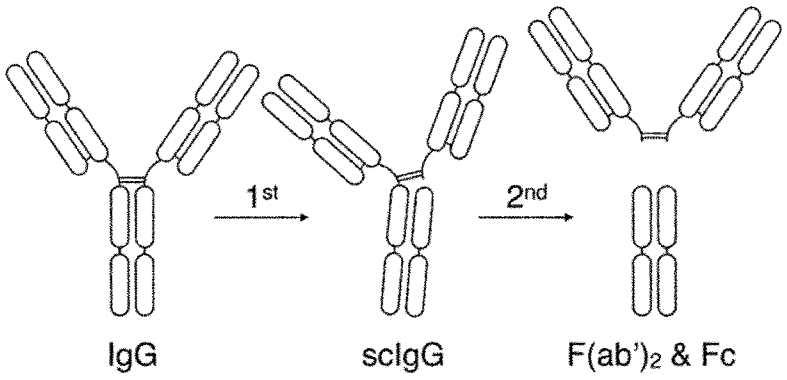
(54) Title: CYSTEINE PROTEASE

FIGURE 18



(57) Abstract: The present invention relates to a novel polypeptide which displays IgG cysteine protease activity, and *in vivo* and *ex vivo* uses thereof. Uses of the polypeptide include methods for the prevention or treatment of diseases and conditions mediated by IgG, and methods for the analysis of IgG.

CYSTEINE PROTEASE

Field of the Invention

The present invention relates to a novel polypeptide which displays IgG cysteine protease activity, and *in vivo* and *ex vivo* uses thereof. Uses of the polypeptide include methods for the prevention or treatment of diseases and conditions mediated by IgG, and methods for the analysis of IgG.

Background of the Invention

IdeS (Immunoglobulin G-degrading enzyme of *S. pyogenes*) is an extracellular cysteine protease produced by the human pathogen *S. pyogenes*. IdeS was originally isolated from a group A *Streptococcus* strain of serotype M1, but the *ides* gene has now been identified in all tested group A *Streptococcus* strains. IdeS has an extraordinarily high degree of substrate specificity, with its only identified substrate being IgG. IdeS catalyses a single proteolytic cleavage in the lower hinge region of the heavy chains of all subclasses of human IgG. IdeS also catalyses an equivalent cleavage of the heavy chains of some subclasses of IgG in various animals. IdeS efficiently cleaves IgG to Fc and $F(ab')_2$ fragments via a two-stage mechanism. In the first stage, one (first) heavy chain of IgG is cleaved to generate a single cleaved IgG (scIgG) molecule with a non-covalently bound Fc molecule. The scIgG molecule is effectively an intermediate product which retains the remaining (second) heavy chain of the original IgG molecule. In the second stage of the mechanism this second heavy chain is cleaved by IdeS to release a $F(ab')_2$ fragment and a homodimeric Fc fragment. These are the products generally observed under physiological conditions. Under reducing conditions the $F(ab')_2$ fragment may dissociate to two Fab fragments and the homodimeric Fc may dissociate into its component monomers.

Summary of the Invention

The IgG cleaving ability of IdeS has been shown to have utility *ex vivo*, for example in methods for production of Fab and Fc fragments, which may be used for the analysis of IgG. See, for example, WO2003051914 and WO2009033670. IdeS has also been shown to have *in vivo* utility as a therapeutic agent, since it is capable of the *in vivo* cleavage of IgG molecules which mediate disease or which are otherwise undesirable. See, for example, WO2003051914, WO2006131347 and WO2013110946. IdeS may be used as a therapy for

any disease or condition wholly or partly mediated by IgG. Many autoimmune diseases are wholly or partly mediated by IgG, as is the acute rejection of donated organs.

However, IdeS is an immunogenic protein. That is, when IdeS is used as a therapeutic agent the immune system of the subject receiving IdeS will often respond to it. The reaction of the immune system to IdeS will typically involve the production of antibodies specific for IdeS. These antibodies may be referred to herein as anti-drug antibodies (ADA) specific for IdeS or "IdeS-specific ADA". The immune response to IdeS in general, and the production of IdeS-specific ADA in particular, may cause two related types of problem. Firstly, the efficacy of IdeS may be reduced, e.g. due to ADA binding, potentially requiring higher or repeat doses to achieve the same effect. ADA which have this effect may be referred to as "neutralising ADA". Secondly, there may be undesirable or even harmful complications, such as a hyper-inflammatory response triggered by immune complexes of ADA and IdeS. The higher the quantity of ADA specific for IdeS in a given subject, the greater the likelihood of these problems. The presence and quantity of IdeS-specific ADA molecules in a patient may be determined by any suitable method, such as an agent specific CAP FEIA (ImmunoCAP) test or a titre assay conducted on a serum sample from the patient. Above a threshold determined by the clinician, the quantity of IdeS-specific ADA molecules in the patient may preclude administration of IdeS, or indicate that a higher dose of IdeS is required. Such a higher dose may in turn result in an increased quantity of IdeS-specific ADA molecules in the patient, thereby precluding further administration of IdeS.

IdeS is a virulence factor of *S. pyogenes*, which is responsible for common infections like tonsillitis and strep throat. Accordingly most human subjects have encountered IdeS in this context and are likely to have anti-IdeS antibodies in the bloodstream. IdeS-specific ADA are routinely detected in serum samples from random human subjects (likely due to prior streptococcal infections), as well as in IVIg (Intravenous Immunoglobulin) preparations, which are preparations of IgG extracted from the pooled serum of thousands of donors. Even if a subject does not possess IdeS-specific ADA prior to an initial administration of IdeS, it is likely that such molecules will be produced subsequently. Thus, for any given subject, the problems associated with the immunogenicity of IdeS are likely to present a barrier to the use of IdeS as a treatment. These problems may require increases to the dose of IdeS and/or preclude treatment with IdeS entirely, particularly if repeat administrations are required. Existing approaches to problems of this type involve, for example, PEGylation of a therapeutic agent to reduce immunogenicity or co-administration of the therapeutic agent with an immune-suppressive agent.

The present inventors have adopted an entirely different approach. The inventors analysed the sequence of IdeS and compared it to the sequence of the protein IdeZ, which has approximately 66% identity to IdeS. IdeZ is an IgG cysteine protease produced by *Streptococcus equi ssp. zooepidemicus*, a bacterium predominantly found in horses. As IdeZ is not a human pathogen, human subjects do not typically have antibodies against this protein in their plasma. However, IdeZ has a level of IgG cysteine protease activity against human IgG which is considerably lower than that of IdeS. The present inventors investigated positions in the sequence of IdeZ which improve its activity against human IgG without resulting in a significant increase in immunogenicity. As starting points for this investigation, the inventors used both the sequence of IdeZ and the sequence of a novel hybrid sequence designed by the inventors, which has 81.7% identity with IdeS and 81% identity with IdeZ. This hybrid sequence may be referred to herein as IdeS/Z.

The full sequence of IdeS is publically available as NCBI Reference Sequence no. WP_010922160.1 and is provided herein as SEQ ID NO: 1. This sequence includes an N terminal methionine followed by a 28 amino acid secretion signal sequence. The N terminal methionine and the signal sequence (a total of 29 amino acids at the N terminus) are typically removed to form the mature IdeS protein, the sequence of which is publically available as Genbank accession no. ADF13949.1 and is provided herein as SEQ ID NO: 2.

The full sequence of IdeZ is publically available as NCBI Reference Sequence no WP_014622780.1 and is provided herein as SEQ ID NO: 3. This sequence includes an N terminal methionine followed by a 33 amino acid secretion signal sequence. The N terminal methionine and the signal sequence (a total of 34 amino acids at the N terminus) are typically removed to form the mature IdeZ protein, the sequence of which is provided herein as SEQ ID NO: 4.

The sequence of the IdeS/Z hybrid designed by the inventors has an N terminal part based on IdeZ, without the N terminal methionine and the signal sequence (a total of 34 amino acids at the N terminus). This sequence is provided herein as SEQ ID NO: 5.

The present inventors have been able to identify positions within the sequence of IdeZ and IdeS/Z hybrid which, when modified as described herein, lead to novel polypeptides which have increased IgG cysteine protease activity against human IgG relative to IdeZ. The IgG cysteine protease activity against human IgG of a polypeptide of the invention is preferably at least as high as the IgG cysteine protease activity against human IgG of IdeS. A polypeptide of the invention may be more effective at cleaving the first chain of an IgG

molecule than the second chain (see schematic representation in Figure 18), particularly when the IgG is an IgG2 isotype. A polypeptide of the invention may be more effective at cleaving IgG1 than IgG2. The polypeptide of the invention is typically less immunogenic than IdeS and may preferably be no more immunogenic than IdeZ or IdeS/Z.

Unless otherwise stated, all references to numbering of amino acid positions in the polypeptides disclosed herein is based on the numbering of the corresponding positions in SEQ ID NO: 3, starting from the N terminus. Thus, since SEQ ID NOs: 4 and 5 lack the N terminal methionine and 33 amino acid signal sequence of SEQ ID NO: 3, the aspartic acid (D) residue at the N terminus of SEQ ID NOs: 4 and 5 is referred to as position 35 as this the corresponding position in SEQ ID NO: 3. Applying this numbering scheme, the most critical residue for IgG cysteine protease activity of IdeS is the cysteine (C) at position 102 (68^{th} residue from the N terminus of SEQ ID NOs: 4 and 5). Other residues likely to be important for IgG cysteine protease activity are the lysine (K) at position 92, the histidine (H) at position 272, and the aspartic acid (D) at each of positions 294 and 296 of SEQ ID NO: 3. These are the 58^{th}, 238^{th}, 260^{th} and 262^{nd} residues from the N terminus of SEQ ID NO: 4 and the 58^{th}, 236^{th}, 258^{th} and 260^{th} from the N terminus of SEQ ID NO: 5, respectively.

In accordance with the present invention, there is thus provided a polypeptide having IgG cysteine protease activity and comprising a variant of the sequence of SEQ ID NO:4 or 5, which variant:

(a) is at least 50% identical to SEQ ID NO: 4 or 5;

(b) has a cysteine (C) at the position in said variant sequence which corresponds to position 102 of SEQ ID NO: 3; and optionally

(c) has, at the positions in said variant sequence which correspond to positions 92, 272, 294 and 296 of SEQ ID NO: 3, a lysine (K), a histidine (H), an aspartic acid (D) and an aspartic acid (D), respectively;

wherein said polypeptide is more effective at cleaving human IgG than IdeZ and/or is at least as effective at cleaving human IgG as IdeS.

Preferably, said variant of SEQ ID NO: 4 or 5:

(1) has a positively charged amino acid at the position in said variant which corresponds to position 138 of SEQ ID NO: 3, optionally wherein said positively charged amino acid is arginine (R) or lysine (K); and/or

(2) has a positively charged amino acid at the position in said variant which corresponds to position 139 of SEQ ID NO: 3, optionally wherein said positively charged amino acid is arginine (R) or lysine (K); and/or

(3) does not include the contiguous sequence DDYQRNATEA YAKEVPHQIT; and/or

(4) has at least one of the following modifications:

i. a deletion of the leucine (L) and threonine (T) residues at the positions in said variant which correspond to positions 64 and 65 of SEQ ID NO: 3;

ii. a threonine (T) in place of the arginine (R) at the position in said variant which corresponds to position 70 of SEQ ID NO: 3;

iii. a deletion of the tyrosine (Y) at the position in said variant which corresponds to position 71 of SEQ ID NO: 3;

iv. a glutamine (Q) in place of the asparagine (N) at the position in said variant which corresponds to position 72 of SEQ ID NO: 3;

v. a glycine (G) in place of the asparagine (N) at the position in said variant which corresponds to position 73 of SEQ ID NO: 3;

vi. a alanine (A) in place of the glutamic acid (E) at the position in said variant which corresponds to position 67 of SEQ ID NO: 3;

vii. a asparagine (N) in place of the glutamine (Q) at the position in said variant which corresponds to position 68 of SEQ ID NO: 3.

The at least one modification of (4) is typically selected from options i. to vii. above. A polypeptide of the invention may comprise a variant of the amino acid sequence of SEQ ID NO: 4 or 5, which variant has at least two, three, four, five, six or all seven of the modifications of options i. to vii.

The invention also provides a polynucleotide, an expression vector or a host cell encoding or expressing a polypeptide of the invention.

The invention also provides a method of treating or preventing a disease or condition mediated by IgG antibodies in a subject, the method comprising administering to the subject a therapeutically or prophylactically effective amount of a polypeptide of the invention. The method may typically comprise multiple administrations of said polypeptide to the subject.

The invention also provides a method of treating, *ex vivo*, blood taken from a patient, typically a patient suffering from a disease or condition mediated by IgG antibodies, which method comprises contacting the blood with a polypeptide of the invention.

The invention also provides a method for improving the benefit to a subject of a therapy or therapeutic agent, the method comprising (a) administering to the subject a polypeptide of the invention; and (b) subsequently administering said therapy or said therapeutic agent to the subject; wherein:

- said therapy is an organ transplant or said therapeutic agent is an antibody, a gene therapy such as a viral vector, a replacement for a defective endogenous factor such as an enzyme, a growth or a clotting factor, or a cell therapy;
- the amount of said polypeptide administered is sufficient to cleave substantially all IgG molecules present in the plasma of the subject; and
- steps (a) and (b) are separated by a time interval which is sufficient to cleave substantially all IgG molecules present in the plasma of the subject.

The invention also provides a method of generating Fc, Fab or $F(ab')_2$ fragments of IgG comprising contacting IgG with a polypeptide of the invention, preferably ex vivo.

Also provided are kits for carrying out the methods according to the invention.

Brief Description of the Figures

Figures 1 and 2 show the results of a representative assay to determine the potency (efficacy at cleavage of IgG) of polypeptides of the invention as compared to controls.

Figure 3 shows the results of a representative SDS-PAGE gel used to visualize the cleavage products produced by incubation of IgG1 with polypeptides of the invention or controls.

Figure 4 shows the results of a representative SDS-PAGE gel used to visualize the cleavage products produced by incubation of IVIg with polypeptides of the invention or controls.

Figure 5 shows the results of a representative SDS-PAGE gel used to visualize the cleavage products produced by incubation of IgG1 with further polypeptides of the invention or controls.

Figure 6 shows the results of a representative SDS-PAGE gel used to visualize the cleavage products produced by incubation of IgG2 with polypeptides of the invention or controls.

Figure 7 shows the results of a representative SDS-PAGE gel used to visualize the cleavage products produced by incubation of IVIg with polypeptides of the invention or controls.

Figures 8 and 9 show the results of representative competition assays to determine the level of recognition of polypeptides of the invention by IdeS-specific antibodies, as compared to controls.

Figures 10 and 11 show the results of representative titration assays to determine the level of recognition of polypeptides of the invention by IdeS-specific antibodies, as compared to controls.

Figure 12 shows representative titration curves for cleavage of IgG1 by different IgG cysteine protease polypeptides.

Figure 13 shows representative titration curves for cleavage of IgG2 by different IgG cysteine protease polypeptides.

Figure 14 shows the result of a representative SDS-PAGE used to visualize the cleavage products produced by incubation IgG with polypeptides of the invention or controls.

Figure 15 shows the results of a representative SDS-PAGE used to visualize the cleavage products produced by incubation of IgG with polypeptides of the invention or controls.

Figure 16 shows the results of a representative SDS-PAGE used to visualize the cleavage products produced by incubation of IVIg with polypeptides of the invention or controls.

Figure 17 shows the results of a representative SDS-PAGE used to visualize the cleavage products produced by incubation of IVIg with polypeptides of the invention or controls.

Figure 18 Schematic representation of the cleavage of immunoglobulins by polypeptides of the invention.

Figure 19 shows the results of a representative % competition of ADA binding sites with polypeptides of the invention or controls.

Figure 20 shows the results of a further representative % competition of ADA binding sites with polypeptides of the invention or controls.

Figure 21 shows the results of a representative efficacy ELISA used to determine the efficacy of the polypeptides of the invention in cleaving human IgG *in vivo*.

Figure 22 shows the results of a representative SDS-PAGE used to visualize the IgG cleavage products produced *in vivo* by polypeptides of the invention.

Brief Description of the Sequences

SEQ ID NO: 1 is the full sequence of IdeS including N terminal methionine and signal sequence. Also disclosed as NCBI Reference sequence no. WP_010922160.1

SEQ ID NO: 2 is the mature sequence of IdeS, lacking the N terminal methionine and signal sequence. Also disclosed as Genbank accession no. ADF13949.1

SEQ ID NO: 3 is the full sequence of IdeZ including N terminal methionine and signal sequence. Also disclosed as NCBI Reference sequence no. WP_014622780.1.

SEQ ID NO: 4 is the mature sequence of IdeZ, lacking the N terminal methionine and signal sequence.

SEQ ID NO: 5 is the sequence of a hybrid IdeS/Z designed by the inventors. The N terminus is based on IdeZ lacking the N terminal methionine and signal sequence.

SEQ ID NOs: 6 to 25 are the sequences of exemplary polypeptides of the invention

SEQ ID NO: 26 is the sequence of an IdeS polypeptide used herein as a control. Comprises the sequence of SEQ ID NO: 2 with an additional N terminal methionine and a histidine tag (internal reference pCART124).

SEQ ID NO: 27 is the sequence of an IdeZ polypeptide used herein as a control. Comprises the sequence of SEQ ID NO: 4 with an additional N terminal methionine and a histidine tag (internal reference pCART144).

SEQ ID NO: 28 is the sequence of an IdeS/Z polypeptide used herein as a control. Comprises the sequence of SEQ ID NO: 5 with an additional N terminal methionine and a histidine tag (internal reference pCART145).

SEQ ID NO: 29 is the contiguous sequence PLTPEQFRYNN, which corresponds to positions 63-73 of SEQ ID NO: 3.

SEQ ID NO: 30 is the contiguous sequence PPANFTQG, which corresponds to positions 58-65 of SEQ ID NO: 1.

SEQ ID NO: 31 is the contiguous sequence DDYQRNATEAYAKEVPHQIT, which corresponds to positions 35-54 of SEQ ID NO: 3.

SEQ ID NO: 32 is the contiguous sequence DSFSANQEIRYSEVTPYHVT, which corresponds to positions 30-49 of SEQ ID NO: 1.

SEQ ID NOs: 33 to 55 are nucleotide sequences encoding polypeptides disclosed herein.

Detailed Description of the Invention

It is to be understood that different applications of the disclosed products and methods may be tailored to the specific needs in the art. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

In addition as used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a polypeptide" includes "polypeptides", and the like.

A "polypeptide" is used herein in its broadest sense to refer to a compound of two or more subunit amino acids, amino acid analogs, or other peptidomimetics. The term "polypeptide" thus includes short peptide sequences and also longer polypeptides and proteins. As used herein, the term "amino acid" refers to either natural and/or unnatural or synthetic amino acids, including both D or L optical isomers, and amino acid analogs and peptidomimetics.

The terms "patient" and "subject" are used interchangeably and typically refer to a human. References to IgG typically refer to human IgG unless otherwise stated.

Functional features of the polypeptide

The present invention relates to a novel polypeptide having IgG cysteine protease activity, wherein said polypeptide is more effective at cleaving human IgG than IdeZ. The IgG cysteine protease activity against human IgG of a polypeptide of the invention is preferably at least as high as the IgG cysteine protease activity against human IgG of IdeS. In addition the polypeptide of the invention is typically less immunogenic than IdeS and may preferably be no more immunogenic than IdeZ or IdeS/Z. In the context of a control or a comparison relative to a polypeptide of the invention, "IdeS", "IdeZ" and "IdeS/Z" refers to a polypeptide consisting of the amino acid sequence of SEQ ID NO: 2, 4 and 5, respectively. Alternatively or in addition, "IdeS", "IdeZ" and "IdeS/Z" when used as a control or a comparison may refer to a polypeptide comprising the sequence the amino acid sequence of SEQ ID NO: 2, 4 and 5, respectively, with an additional methionine (M) residue at the N terminus and/or a tag at the C terminus to assist with expression in and isolation from standard bacterial expression systems. Suitable tags include a histidine tag which may be joined directly to the C terminus of a polypeptide or joined indirectly by any suitable linker sequence, such as 3, 4 or 5 glycine residues. The histidine tag typically consists of six histidine residues, although it can be longer than this, typically up to 7, 8, 9, 10 or 20 amino acids or shorter, for example 5, 4, 3, 2 or 1 amino acids. The sequence of an exemplary IdeS polypeptide used herein is a control is provided as SEQ ID NO: 22. This polypeptide comprises the sequence of SEQ ID NO: 2 with an additional N terminal methionine and a histidine tag and may be referred to herein as pCART124. The sequence of an exemplary IdeZ polypeptide used herein is a control is provided as SEQ ID NO: 23. This polypeptide comprises the sequence of SEQ ID NO: 4 with an additional N terminal methionine and a histidine tag and may be referred to herein as pCART144. The sequence of an exemplary IdeS/Z polypeptide used herein is a control is provided as SEQ ID NO: 24. This polypeptide comprises the sequence of SEQ ID NO: 5 with an additional N terminal methionine and a histidine tag and may be referred to herein as pCART145.

IgG cysteine protease activity may be assessed by any suitable method, for example by incubating a polypeptide with a sample containing IgG and determining the presence of

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IgG cleavage products. Efficacy may be assessed in the presence or absence of an inhibitor, such as a neutralising antibody. However, efficacy herein will typically mean efficacy as assessed in the absence of such an inhibitor unless otherwise stated. Suitable methods are described in the Examples. The efficacy of a polypeptide at cleavage of IgG may be referred to herein as the "potency" of the polypeptide. The potency of a polypeptide of the invention is preferably at least 2.0 fold greater than the potency of IdeZ measured in the same assay. Alternatively, the potency of a polypeptide of the invention is preferably at least equivalent to the potency of IdeS measured in the same assay. The potency of a polypeptide of the invention may be at least 1.5 fold, 2.0 fold, 2.5 fold, 3.0 fold, 4.0 fold, 4.5 fold, 5.0 fold, 6.0 fold, 7.0 fold, 7.5 fold or 8.0 fold greater than the potency of IdeS measured in the same assay. The potency of a polypeptide of the invention is preferably at least 2.0 fold, more preferably at least 3.0 or 4.0 fold and most preferably at least 8.0 fold greater than the potency of IdeS measured in the same assay.

The polypeptide of the invention is typically less immunogenic than IdeS and so increased potency relative to that of IdeZ and/or potency equivalent to that of IdeS is an acceptable minimum standard for cysteine protease activity against human IgG. However, increased potency relative to IdeS is a desirable improvement. Such increased potency will typically enable the use of a lower dose of a polypeptide of the invention for the same therapeutic effect as a higher dose of IdeS. The lower dose may also permit a greater number of repeat administrations of a polypeptide of the invention relative to IdeS. This is because the use of a lower dose reduces the problems associated with immunogenicity of a therapeutic agent, because the immune system is less likely to respond, or will respond less vigorously, to an agent which is present at a lower concentration.

Assays for assessing the efficacy of a polypeptide at the cleavage of IgG, that is assays for assessing the potency of a polypeptide, are well known in the art and any suitable assay may be used. Suitable assays include an ELISA-based assay, such as that which is described in the Examples. In such an assay, the wells of an assay plate will typically be coated with an antibody target, such as bovine serum albumin (BSA). Samples of the polypeptide to be tested are then added to the wells, followed by samples of target-specific antibody that is antibody specific for BSA in this example. The polypeptide and antibody are allowed to interact under conditions suitable for IgG cysteine protease activity. After a suitable interval, the assay plate will be washed and a detector antibody which specifically binds to the target-specific antibody will be added under conditions suitable for binding to the target-specific antibody. The detector antibody will bind to any intact target-specific

antibody that has bound to the target in each well. After washing, the amount of detector antibody present in a well will be proportional to the amount of target-specific antibody bound to that well. The detector antibody may be conjugated directly or indirectly to a label or another reporter system (such as an enzyme), such that the amount of detector antibody remaining in each well can be determined. The higher the potency of the tested polypeptide that was in a well, the less intact target-specific antibody will remain and thus there will be less detector antibody. Typically, at least one well on a given assay plate will include IdeS instead of a polypeptide to be tested, so that the potency of the tested polypeptides may be directly compared to the potency of IdeS. IdeZ and IdeS/Z may also be included for comparison.

Other assays may determine the potency of a tested polypeptide by directly visualizing and/or quantifying the fragments of IgG which result from cleavage of IgG by a tested polypeptide. An assay of this type is also described in the Examples. Such an assay will typically incubate a sample of IgG with a test polypeptide (or with one or more of IdeS, IdeZ and IdeS/Z as a control) at differing concentrations in a titration series. The products which result from incubation at each concentration are then separated using gel electrophoresis, for example by SDS-PAGE. Whole IgG and the fragments which result from cleavage of IgG can then be identified by size and quantified by the intensity of staining with a suitable dye. The greater the quantity of cleavage fragments, the greater the potency of a tested polypeptide at a given concentration. A polypeptide of the invention will typically produce detectable quantities of cleavage fragments at a lower concentration (a lower point in the titration series) than IdeZ and/or IdeS. This type of assay may also enable the identification of test polypeptides that are more effective at cleaving the first or the second heavy chain of an IgG molecule, as the quantities of the different fragments resulting from each cleavage event may also be determined. A polypeptide of the invention may be more effective at cleaving the first chain of an IgG molecule than the second chain (see schematic representation in Figure 18), particularly when the IgG is an IgG2 isotype. A polypeptide of the invention may be more effective at cleaving IgG1 than IgG2.

This type of assay may also be adapted to determine the extent to which the presence of IdeS-specific ADA may reduce the potency of a polypeptide of the invention. In the adapted assay, when a sample of IgG is incubated with a test polypeptide (or with IdeS as a control), serum or an IVIg preparation containing IdeS-specific ADA is included with the reaction medium. Preferably, the potency of a polypeptide of the invention is not affected by the presence of ADA or is less reduced by the presence of ADA than the potency of IdeS in

the same assay. In other words, preferably the neutralizing effect of IdeS-specific ADA on the polypeptide of the invention is the same or lower than the neutralizing effect of IdeS-specific ADA on IdeS, measured in the same assay.

As indicated above, a polypeptide of the invention is typically less immunogenic than IdeS. That is, a polypeptide of the invention may result in the same or preferably a lower immune response than IdeS when present at an equivalent dose or concentration and measured in the same assay. The immunogenicity of a polypeptide of the invention is typically no more than 50%, no more than 45%, no more than 40%, no more than 35%, no more than 30%, or no more than 25% of the immunogenicity of IdeS measured in the same assay. Preferably the immunogenicity of a polypeptide of the invention is no more than 25% of the immunogenicity of IdeS measured in the same assay.

Assays for assessing the immunogenicity of a polypeptide are also well known in the art and any suitable assay may be used. Preferred assays for assessing the immunogenicity of a polypeptide relative to the immunogenicity of IdeS involves assessing the extent to which ADA specific for IdeS also bind to a polypeptide of the invention. Assays of this type are described in the Examples.

One such an assay involves testing for competition between IdeS and a test polypeptide for binding to IdeS-specific ADA. Typically, the wells of an assay plate are coated with IdeS, followed by administration of a pre-incubated mixture of a solution containing IdeS-specific ADA, e.g. an IVIg preparation, and a test polypeptide (or IdeS as a control). The pre-incubation takes place in the presence of an inhibitor of IgG cysteine protease activity, e.g. iodoacetic acid (IHAc), and at high salt concentration so that only high affinity binding between protein and ADA is permitted. The pre-incubated mixture is allowed to interact with the IdeS coated wells. Any IdeS-specific ADA not bound to test polypeptide will bind to the IdeS on the wells. After a suitable interval, the assay plate will be washed and a detector antibody which specifically binds to IgG will be added under conditions suitable for binding. The detector antibody will bind to any ADA that has bound to the IdeS in each well. After washing, the amount of detector antibody present in a well will be inversely proportional to the amount of ADA that had bound to the test polypeptide. The detector antibody may be conjugated directly or indirectly to a label or another reporter system (such as an enzyme), such that the amount of detector antibody remaining in each well can be determined. Typically, at least one well on a given assay plate will be tested with a pre-incubated mixture of IVIg and IdeS instead of a polypeptide to be tested, so that the

binding of ADA to the tested polypeptides may be directly compared to the binding to IdeS, IdeZ and/or IdeS/Z may also be included as further controls.

Another suitable assay involves testing the extent to which a titration series of different concentrations of IdeS-specific ADA, e.g. an IVIg preparation, binds to a test polypeptide as compared to IdeS and/or IdeZ as control. Preferably, a polypeptide of the invention will require a higher concentration of ADA for binding to be detectable, relative to the concentration of ADA for which binding to IdeS is detectable. Such an assay is described in the Examples. Such an assay typically involves coating the wells of an assay plate with test polypeptide or control, followed by incubating with each well with a different concentration of IdeS-specific ADA from a titration series. The incubations are conducted in the presence of an inhibitor of IgG cysteine protease activity, e.g. iodoacetic acid (IHAc), and at high salt concentration so that only high affinity binding between protein and ADA is permitted. After a suitable interval, the assay plate will be washed and a detector antibody which specifically binds to IgG $F(ab')_2$ will be added under conditions suitable for binding. The detector antibody will bind to any ADA that has bound to the test polypeptide or the IdeS in each well. After washing, the amount of detector antibody present in a well will be directly proportional to the amount of ADA that had bound to the test polypeptide or control. The detector antibody may be conjugated directly or indirectly to a label or another reporter system (such as an enzyme), such that the amount of detector antibody remaining in each well can be determined. At least one well on a given assay plate will be incubated with buffer lacking ADA as a blank to establish a threshold level for detection of binding in the test wells.

Structural features of the polypeptide

This section sets out the structural features of a polypeptide of the invention, which apply in addition to the functional features outlined in the preceding section.

The polypeptide of the invention is typically at least 100, 150, 200, 250, 260, 270, 280, 290, 300 or 310 amino acids in length. The polypeptide of the invention is typically no larger than 400, 350, 340, 330, 320 or 315 amino acids in length. It will be appreciated that any of the above listed lower limits may be combined with any of the above listed upper limits to provide a range for the length the polypeptide of the invention. For example, the polypeptide may be 100 to 400 amino acids in length, or 250 to 350 amino acids in length. The polypeptide is preferably 290 to 320 amino acids in length, most preferably 300 to 315 amino acids in length.

The primary structure (amino acid sequence) of a polypeptide of the invention is based on the primary structure of IdeZ or IdeS/Z, specifically the amino acid sequence of SEQ ID NO: 4 or 5, respectively. The sequence of a polypeptide of the invention comprises a variant of the amino acid sequence of SEQ ID NO: 4 or 5, which is at least 50% identical to the amino acid sequence of SEQ ID NO: 4 or 5. The variant sequence may be at least 60%, at least 70%, at least 80%, at least, 85%, preferably at least 90%, at least 95%, at least 98% or at least 99% identical to the sequence of SEQ ID NO: 4 or 5. The variant may be identical to the sequence of SEQ ID NO: 4 or 5 apart from the inclusion of one or more of the specific modifications identified herein. Identity relative to the sequence of SEQ ID NO: 4 or 5 can be measured over a region of at least 50, at least 100, at least 200, at least 300 or more contiguous amino acids of the sequence shown in SEQ ID NO: 4 or 5, or more preferably over the full length of SEQ ID NO: 4 or 5.

Amino acid identity may be calculated using any suitable algorithm. For example the PILEUP and BLAST algorithms can be used to calculate identity or line up sequences (such as identifying equivalent or corresponding sequences (typically on their default settings), for example as described in Altschul S. F. (1993) J Mol Evol 36:290-300; Altschul, S, F *et al* (1990) J Mol Biol 215:403-10. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul *et al*, supra). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extensions for the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) *Proc. Natl. Acad. Sci.* USA 89: 10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g., Karlin and Altschul (1993) *Proc. Natl. Acad. Sci.* USA 90: 5873-5787. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two polynucleotide or amino acid sequences would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001. Alternatively, the UWGCG Package provides the BESTFIT program which can be used to calculate identity (for example used on its default settings) (Devereux *et al* (1984) *Nucleic Acids Research* 12, 387-395).

The sequence of a polypeptide of the invention comprises a variant of the amino acid sequence of SEQ ID NO: 4 or 5 in which modifications, such as amino acid additions, deletions or substitutions are made relative to the sequence of SEQ ID NO: 4 or 5. Unless otherwise specified, the modifications are preferably conservative amino acid substitutions. . Conservative substitutions replace amino acids with other amino acids of similar chemical structure, similar chemical properties or similar side-chain volume. The amino acids introduced may have similar polarity, hydrophilicity, hydrophobicity, basicity, acidity, neutrality or charge to the amino acids they replace. Alternatively, the conservative substitution may introduce another amino acid that is aromatic or aliphatic in the place of a pre-existing aromatic or aliphatic amino acid. Conservative amino acid changes are well-known in the art and may be selected in accordance with the properties of the 20 main amino acids as defined in Table A1 below. Where amino acids have similar polarity, this can be determined by reference to the hydropathy scale for amino acid side chains in Table A2.

Table A1 - Chemical properties of amino acids

Ala (A)	aliphatic, hydrophobic, neutral	Met (M)	hydrophobic, neutral
Cys (C)	polar, hydrophobic, neutral	Asn (N)	polar, hydrophilic, neutral
Asp (D)	polar, hydrophilic, charged (-)	Pro (P)	hydrophobic, neutral
Glu (E)	polar, hydrophilic, charged (-)	Gln (Q)	polar, hydrophilic, neutral
Phe (F)	aromatic, hydrophobic, neutral	Arg (R)	polar, hydrophilic, charged (+)
Gly (G)	aliphatic, neutral	Ser (S)	polar, hydrophilic, neutral
His (H)	aromatic, polar, hydrophilic, charged (+)	Thr (T)	polar, hydrophilic, neutral
Ile (I)	aliphatic, hydrophobic, neutral	Val (V)	aliphatic, hydrophobic, neutral
Lys (K)	polar, hydrophilic, charged(+)	Trp (W)	aromatic, hydrophobic, neutral
Leu (L)	aliphatic, hydrophobic, neutral	Tyr (Y)	aromatic, polar, hydrophobic

Table A2 - Hydropathy scale

Side Chain	Hydropathy
Ile	4.5
Val	4.2
Leu	3.8
Phe	2.8
Cys	2.5
Met	1.9
Ala	1.8
Gly	-0.4
Thr	-0.7
Ser	-0.8
Trp	-0.9
Tyr	-1.3
Pro	-1.6
His	-3.2
Glu	-3.5
Gln	-3.5
Asp	-3.5
Asn	-3.5
Lys	-3.9
Arg	-4.5

The amino acid sequence of a polypeptide of the invention comprises a variant of the amino acid sequence of SEQ ID NO: 4 or 5. However, certain residues in the amino acid sequence of SEQ ID NO: 4 or 5 are preferably retained within the said variant sequence. For example, the said variant sequence typically retains certain residues which are known to be required for IgG cysteine protease activity. Thus, the cysteine at position 102 of SEQ ID NO: 3 must be retained (68th residue of SEQ ID NO: 4 or 5) in the amino acid sequence of a polypeptide of the invention. Optionally, the lysine (K) at position 92, the histidine (H) at position 272, and the aspartic acid (D) at each of positions 294 and 296 of SEQ ID NO: 3.are also retained. These are the 58th, 238th, 260th and 262nd residues from the N terminus of SEQ ID NO: 4 and the 58th, 236th, 258th and 260th from the N terminus of SEQ ID NO: 5, respectively. Thus, a polypeptide of the invention typically comprises a variant of the amino acid sequence of SEQ ID NO: 2 which has a cysteine (C) at the position in said variant sequence which corresponds to position 102 of SEQ ID NO: 3; and optionally has, at the positions in said variant sequence which correspond to positions 92, 272, 294 and 296 of SEQ ID NO: 3, a lysine (K), a histidine (H), an aspartic acid (D) and an aspartic acid (D), respectively.

Starting with the above structural limitations, the inventors identified specific positions for modification to adjust the functional properties of IdeS by assessing a three dimensional model of IdeS. The inventors have identified that:

(1) Replacing the asparagine (N) at position 138 of SEQ ID NO: 3 with a positively charged amino acid enhances the potency of a polypeptide which incorporates this change. Thus, a polypeptide of the invention may comprise a variant of the amino acid sequence of

SEQ ID NO: 4 or 5 which has a positively charged amino acid at the position in said variant which corresponds to position 138 of SEQ ID NO: 3. Common positively charged amino acids are identified in Table A1 above. The positively charged amino acid is preferably arginine (R) or lysine (K). Accordingly this particular modification may be identified herein by the term "N138R/K".

(2) Replacing the asparagine (N) at position 139 of SEQ ID NO: 3 with a positively charged amino acid enhances the potency of a polypeptide which incorporates this change. Thus, a polypeptide of the invention may comprise a variant of the amino acid sequence of SEQ ID NO: 4 or 5 which has a positively charged amino acid at the position in said variant which corresponds to position 139 of SEQ ID NO: 3. Common positively charged amino acids are identified in Table A above. The positively charged amino acid is preferably arginine (R) or lysine (K). Accordingly this particular modification may be identified herein by the term "N139R/K".

(3) Deleting the first twenty residues at the N terminus of SEQ ID NO: 3 may enhance the potency of a polypeptide which incorporates this change and/or may reduce immunogenicity without adversely affecting potency. The first twenty residues at the N terminus of SEQ ID NO: 3 consist of the contiguous sequence DDYQRNATEAYAKEVPHQIT. Thus, a polypeptide of the invention may comprise a variant of the amino acid sequence of SEQ ID NO: 4 or 5 which does not include the contiguous sequence DDYQRNATEAYAKEVPHQIT. That is, the first twenty residues at the N terminus of SEQ ID NO: 4 or 5 may be absent from said variant of SEQ ID NO: 4 or 5. The first twenty residues of SEQ ID NOs: 4 and 5 correspond to positions 35-54 of SEQ ID NO: 3. Accordingly this particular modification may be identified herein by the term "D35_T54del".

(4) The region which corresponds to positions 63 – 73 of SEQ ID NO: 3 is important for the IgG cysteine protease activity of a polypeptide of the invention. Modifications in this region primarily improve the ability of the polypeptide to cleave the second IgG heavy chain, but they also enhance cleavage of the first IgG heavy chain. Specifically, modifying one or more residues within this region in favour of the corresponding residue (or an amino acid with similar characteristics to the corresponding residue) in the equivalent region of IdeS increases the potency of a polypeptide of the invention. The equivalent region in IdeS corresponds to positions 58 – 65 of SEQ ID NO: 1. The following alignment shows positions 63-73 of SEQ ID NO: 3 alongside positions 58-65 of SEQ ID NO: 1.

^{63}PLTPEQFRYNN^{73} (region of IdeZ, SEQ ID NO: 3)

^{58}P--PANFT-QG^{65} (region of IdeS, SEQ ID NO: 1)

"-" indicates absent residue

Thus, a polypeptide of the invention may comprise a variant of the amino acid sequence of SEQ ID NO: 4 or 5, which variant may have at least one of the following modifications:

i. a deletion of the leucine (L) and threonine (T) residues at the positions in said variant which correspond to positions 64 and 65 of SEQ ID NO: 3;

ii. a threonine (T) in place of the arginine (R) at the position in said variant which corresponds to position 70 of SEQ ID NO: 3;

iii. a deletion of the tyrosine (Y) at the position in said variant which corresponds to position 71 of SEQ ID NO: 3;

iv. a glutamine (Q) in place of the asparagine (N) at the position in said variant which corresponds to position 72 of SEQ ID NO: 3;

v. a glycine (G) in place of the asparagine (N) at the position in said variant which corresponds to position 73 of SEQ ID NO: 3;

vi. a alanine (A) in place of the glutamic acid (E) at the position in said variant which corresponds to position 67 of SEQ ID NO: 3;

vii. a asparagine (N) in place of the glutamine (Q) at the position in said variant which corresponds to position 68 of SEQ ID NO: 3.

The at least one modification from options i. to vii. above is typically selected from options i. to v. A polypeptide of the invention may comprise a variant of the amino acid sequence of SEQ ID NO: 4 or 5, which variant has at least two, three, four, or all five of the modifications of i. to v. Preferably, said variant has at least one, two, three of all four of modifications ii. to v., and optionally modification i. is also present. In a particularly preferred said variant, all of modifications i. to v. are present.

In summary therefore, a polypeptide of the invention comprises a variant of the sequence of SEQ ID NO: 4 or 5, which variant:

(a) is at least 50% identical to SEQ ID NO: 4 or 5;

(b) has a cysteine (C) at the position in said variant sequence which corresponds to position 102 of SEQ ID NO: 3; and optionally

(c) has, at the positions in said variant sequence which correspond to positions 92, 272, 294 and 296 of SEQ ID NO: 3, a lysine (K), a histidine (H), an aspartic acid (D) and an aspartic acid (D), respectively.

Preferably, said variant of SEQ ID NO: 4 or 5:

(1) has a positively charged amino acid at the position in said variant which corresponds to position 138 of SEQ ID NO: 3, optionally wherein said positively charged amino acid is arginine (R) or lysine (K); and/or

(2) has a positively charged amino acid at the position in said variant which corresponds to position 139 of SEQ ID NO: 3, optionally wherein said positively charged amino acid is arginine (R) or lysine (K); and/or

(3) does not include the contiguous sequence DDYQRNATEA YAKEVPHQIT; and/or

(4) has at least one of the following modifications:

i. a deletion of the leucine (L) and threonine (T) residues at the positions in said variant which correspond to positions 64 and 65 of SEQ ID NO: 3;

ii. a threonine (T) in place of the arginine (R) at the position in said variant which corresponds to position 70 of SEQ ID NO: 3;

iii. a deletion of the tyrosine (Y) at the position in said variant which corresponds to position 71 of SEQ ID NO: 3;

iv. a glutamine (Q) in place of the asparagine (N) at the position in said variant which corresponds to position 72 of SEQ ID NO: 3;

v. a glycine (G) in place of the asparagine (N) at the position in said variant which corresponds to position 73 of SEQ ID NO: 3;

vi. a alanine (A) in place of the glutamic acid (E) at the position in said variant which corresponds to position 67 of SEQ ID NO: 3;

vii. a asparagine (N) in place of the glutamine (Q) at the position in said variant which corresponds to position 68 of SEQ ID NO: 3.

wherein the at least one modification of (4) is typically selected from options i. to v, and wherein preferably all of options ii. to v. are present optionally also with option i.

A polypeptide of the invention typically comprises a variant of the amino acid sequence of SEQ ID NO: 4 or 5 which variant includes at least one, two, three or all four of modifications (1) to (4) set out above. Said variant may include any combination of two or three of the modifications of (1) to (4). A preferred variant includes modification (3) and at least one of modifications (1) and (2). Alternatively, the variant may include none of the modifications of (1) to (3) set out above.

The inventors have also determined that certain other modifications to the sequence of SEQ ID NO: 4 or 5, which may be applied alternatively or in addition to any combination of the modifications described above, may increase the potency of a polypeptide of the invention and/or may reduce the recognition of a polypeptide of the invention by IdeS-

specific ADA. Thus, alternatively or in addition to the modifications set out above, the polypeptide of the invention may comprise:

(A) a variant of the sequence of SEQ ID NO: 4 in which a substitution is made at one or more of the positions corresponding to positions 84, 93, 95, 97, 137, 140, 147, 150, 162, 165, 166, 171, 174, 205, 226, 237, 239, 243, 250, 251, 254, 255, 282, 288, 312, 315, 347, 349 of SEQ ID NO: 3, and/or in which the contiguous sequence corresponding to positions 36 to 53 of SEQ ID NO: 3 is replaced with the contiguous sequence at positions 31 to 48 of SEQ ID NO: 2 (this change may be referred to as "D36_I53replacedS31_V48 of SEQ 2");

or

(B) a variant of the sequence of SEQ ID NO: 5 in which a substitution is made at one or more of the positions corresponding to positions 77, 93, 95, 99, 140, 141, 147, 150, 162, 171, 174, 175, 176, 177, 206, 224, 237, 241, 242, 245, 246, 249,253, 267, 280, 286, 310, 311, 313, 344, 345, 346, 347.

The said variant (A) may comprise a substitution in all of the listed positions, or any combination of one or more of the listed positions, but typically comprises a substitution in no more than twelve, eleven or ten of these positions.

The said variant (B) may comprise a substitution in all of the listed positions, or any combination of one or more of the listed positions, but typically comprises a substitution in no more than thirty of these positions.

The substitutions typically replace the existing amino acid with another amino acid that has different properties. For example, an uncharged amino acid may be replaced with a charged amino acid, and vice versa. Preferred substitutions at these positions are set out in Table B1 and B2 below using the one letter code:

Table B1 – variant A

Existing amino acid in SEQ ID NO: 4	Position in SEQ ID NO: 3	Preferred replacement
H	84	N
A	93	T
D	95	N
K	97	A
F	137	I
Q	140	E
A	147	E
D	150	R
N	162	E
R	165	K
D	166	E
N	171	Y
A	174	T
N	205	K
D	226	N
L	237	F

N	239	E
N	243	K
K	250	S
Q	251	E
T	254	E
E	255	K
N	282	D
E	288	K
A	312	K
H	315	K
K	347	Q
S	349	N

Table B2 – variant B

Existing amino acid in SEQ ID NO: 5	Position in SEQ ID NO: 3	Preferred replacement
F	77	I
A	93	T
D	95	N
N	99	D
D	140	E
N	141	Q
K	147	E
D	150	R
N	162	E
G	171	Y
A	174	T
R	175	K
R	176	H
I	177	L
E	206	K
D	224	N
N	237	E
N	241	K
D	242	E
T	245	D
I	246	L
Q	249	E
K	253	E
S	267	R
N	280	D
E	286	K
A	310	K
H	311	A
H	313	K
Q	344	N
K	345	Q
L	346	T
S	347	N

Each of the substitutions in tables B1 and B2 may be referred to herein using a term obtained by combining the entries in the first, second and third columns for each row from left to right. For example, the substitution in the first row of table B1 may be referred to herein as "H84N", the substitution in the second row may be referred to as "A93T", and so on. The

specific modification "D226N" in Table B1 and "D224N" in Table B2 is intended to disrupt a known cell adhesion motif in the sequence of IdeZ and IdeS/Z, which is the contiguous RGD sequence at positions 224-226 of SEQ ID NO: 3.

Table C1 and C2 below summarize the modifications made to produce the amino acid sequences of certain exemplary polypeptides of the invention.

Table C1

Internal reference	Modifications relative to IdeZ (SEQ ID NO: 4) (positions correspond to SEQ ID NO: 3)	SEQ ID NO of full sequence
pCART197	H84N, N138R, A147E,D150R, N162E, N171Y, N205K, D226N, Q251E, E255K, A312K, S349N	6
pCART198	A93T, D95N, Q140E, R165K, D166E, A174T, D226N, L237F, N239E, N243K, N282D, E288K, H315K, K347Q	7
pCART200	D36_I53replacedS31_V48 of SEQ 2 i.e. SFSANQEI RYSEVTPYHV replaces DYQRNATE AYAKEVPHQI	8
pCART201	D35_T54del	9
pCART202	R70T, Y71del, N72Q, N73G	10
pCART203	L64_T65del, R70T, Y71del, N72Q, N73G	11
pCART204	R70T, Y71del	12
pCART206	L64_T65del, R70T, Y71del, N72Q, N73G, F137I	13
pCART207	L64_T65del, R70T, Y71del, N72Q, N73G, N138R	14
pCART208	L64_T65del, R70T, Y71del, N72Q, N73G, F137I, N138R	15
pCART210	L64_T65del, R70T, Y71del, N72Q, N73G, H84N, N138R, N162E, N205K, D226N	16
pCART217	D35_T54del, L64_T65del, R70T, Y71del, N72Q, N73G, N138R, D226N	17
pCART219	L64_T65del, R70T, Y71del, N72Q, N73G, K97A, N138R, D226N	18
pCART226	D35_T54del, L64_T65del, R70T, Y71del, N72Q, N73G, K97A, N138R, D226N	19
pCART229	L64_T65del, R70T, Y71del, N72Q, N73G, N138R, D226N	20

Table C2

Internal reference	Modifications relative to IdeS/Z (SEQ ID NO: 5) (positions correspond to SEQ ID NO: 3)	SEQ ID NO of full sequence
pCART191	R70T, Y71del, N72Q, N73G, D140E, G171Y, R175K, R176H, I177L, S267R	21
pCART192	R70T, Y71del, N72Q, N73G, N138R, D140E, K147E, D150R, N162E, G171Y, R175K, R176H, I177L, E206K, Q249S, K253N, S267R, A310K, S347N	22
pCART193	R70T, Y71del, N72Q, N73G, A93T, D95N, N99D, D140E, N141Q, K147E, N162E, G171Y, A174T, R175K, R176H, I177L, N237E, N241K, D242E, T245D, I246L, K253E, S267R, E286K, H311A, H313K, Q344N, K345Q, L346T	23
pCART194	R70T, Y71del, N72Q, N73G, A93T, D95N, N99D, N138R, D140E, N141Q, K147E, D150R, N162E, G171Y, A174T, R175K, R176H, I177L, E206K, N237E, N241K, D242E, T245D, I246L, Q249E, S267R, N280D, E286K, A310K, H311A, H313K, Q344N, K345Q, L346T, S347N	24
pCART205	L64_65del, R70T, Y71del, N72Q, N73G, F77I, N138R, D140E, N141Q	25

The amino acid sequence of each of SEQ ID NOs: 1 to 5 is reproduced in full below, followed by the amino acid sequence of each of the exemplary polypeptides of the invention described in Tables C1 and C2.

SEQ ID NO: 1
MRKRCYSTSAAVLAAVTLFVLSVDRGVIADSFSANQEIRYSEVTPYHVTSVWTKGVTPPANFTQGEDVFHAPYVA
NQGWYDITKTFNGKDDLLCGAATAGNMLHWWFDQNKDQIKRYLEEHPEKQKINFNGEQMFDVKEAIDTKNHQLDS
KLFEYFKEKAFPYLSTKHLGVFPDHVIDMFINGYRLSLTNHGPTPVKEGSKDPRGGIFDAVFTRGDQSKLLTSRH
DFKEKNLKEISDLIKKELTEGKALGLSHTYANVRINHVINLWGADFDSNGNLKAIYVTDSDSNASIGMKKYFVGV
NSAGKVAISAKEIKEDNIGAQVLGLFTLSTGQDSWNQTN

SEQ ID NO: 2
DSFSANQEIRYSEVTPYHVTSVWTKGVTPPANFTQGEDVFHAPYVANQGWYDITKTFNGKDDLLCGAATA
GNMLHWWFDQNKDQIKRYLEEHPEKQKINFNGEQMFDVKEAIDTKNHQLDSKLFEYFKEKAFPYLSTKHL
GVFPDHVIDMFINGYRLSLTNHGPTPVKEGSKDPRGGIFDAVFTRGDQSKLLTSRHDFKEKNLKEISDLI
KKELTEGKALGLSHTYANVRINHVINLWGADFDSNGNLKAIYVTDSDSNASIGMKKYFVGVNSAGKVAIS
AKEIKEDNIGAQVLGLFTLSTGQDSWNQTN

SEQ ID NO: 3
MKTIAYPNKPHSLSAGLLTAIAIFSLASSNITYADDYQRNATEAYAKEVPHQITSVWTKGVTPLTPEQFRYNNED
VIHAPYLAHQGWYDITKAFDGKDNLLCGAATAGNMLHWWFDQNKTEIEAYLSKHPEKQKIIFNNQELFDLKAAID
TKDSQTNSQLFNYFRDKAFPNLSARQLGVMPDLVLDMFINGYYLNVFKTQSTDVNRPYQDKDKRGGIFDAVFTRG
DQTTLLTARHDLKNKGLNDISTIIKQELTEGRALALSHTYANVSISHVINLWGADFNAEGNLEAIYVTDSDANAS
IGMKKYFVGINAHGHVAISAKKIEGENIGAQVLGLFTLSSGKDIWQKLS

SEQ ID NO: 4
DDYQRNATEAYAKEVPHQITSVWTKGVTPLTPEQFRYNNEDVIHAPYLAHQGWYDITKAFDGKDNLLCGAATAGN
MLHWWFDQNKTEIEAYLSKHPEKQKIIFNNQELFDLKAAIDTKDSQTNSQLFNYFRDKAFPNLSARQLGVMPDLV
LDMFINGYYLNVFKTQSTDVNRPYQDKDKRGGIFDAVFTRGDQTTLLTARHDLKNKGLNDISTIIKQELTEGRAL
ALSHTYANVSISHVINLWGADFNAEGNLEAIYVTDSDANASIGMKKYFVGINAHGHVAISAKKIEGENIGAQVLG
LFTLSSGKDIWQKLS

SEQ ID NO: 5
DDYQRNATEAYAKEVPHQITSVWTKGVTPLTPEQFRYNNEDVFHAPYVANQGWYDITKAFDGKDNLLCGAATAGN
MLHWWFDQNKDQIKRYLEEHPEKQKINFNGDNMFDVKKAIDTKNHQLDSKLFNYFKEKAFPGLSARRIGVFPDHV
IDMFINGYRLSLTNHGPTPVKEGSKDPRGGIFDAVFTRGNQSKLLTSRHDFKNKNLNDISTIIKQELTKGKALGL
SHTYANVSINHVINLWGADFNAEGNLEAIYVTDSDSNASIGMKKYFVGVNAHGHVAISAKKIEGENIGAQVLGLF
TLSTGQDSWQKLS

SEQ ID NO: 6 (pCART197)
DDYQRNATEAYAKEVPHQITSVWTKGVTPLTPEQFRYNNEDVIHAPYLANQGWYDITKAFDGKDNLLCGAATAGN
MLHWWFDQNKTEIEAYLSKHPEKQKIIFRNQELFDLKEAIRTKDSQTNSQLFEYFRDKAFPYLSARQLGVMPDLV
LDMFINGYYLNVFKTQSTDVKRPYQDKDKRGGIFDAVFTRGNQTTLLTARHDLKNKGLNDISTIIKEELTKGRAL
ALSHTYANVSISHVINLWGADFNAEGNLEAIYVTDSDANASIGMKKYFVGINKHGHVAISAKKIEGENIGAQVLG
LFTLSSGKDIWQKLN

SEQ ID NO: 7 (pCART198)
DDYQRNATEAYAKEVPHQITSVWTKGVTPLTPEQFRYNNEDVIHAPYLAHQGWYDITKTFNGKDNLLCGAATAGN
MLHWWFDQNKTEIEAYLSKHPEKQKIIFNNEELFDLKAAIDTKDSQTNSQLFNYFKEKAFPNLSTRQLGVMPDLV
LDMFINGYYLNVFKTQSTDVNRPYQDKDKRGGIFDAVFTRGNQTTLLTARHDFKEKGLKDISTIIKQELTEGRAL
ALSHTYANVSISHVINLWGADFDAEGNLKAIYVTDSDANASIGMKKYFVGINAHGKVAISAKKIEGENIGAQVLG
LFTLSSGKDIWQQLS

SEQ ID NO: 8 (pCART200)
DSFSANQEIRYSEVTPYHVTSVWTKGVTPLTPEQFRYNNEDVIHAPYLAHQGWYDITKAFDGKDNLLCGAATAGN
MLHWWFDQNKTEIEAYLSKHPEKQKIIFNNQELFDLKAAIDTKDSQTNSQLFNYFRDKAFPNLSARQLGVMPDLV

LDMFINGYYLNVFKTQSTDVNRPYQDKDKRGGIFDAVFTRGDQTTLLTARHDLKNKGLNDISTIIKQELTEGRAL
ALSHTYANVSISHVINLWGADFNAEGNLEAIYVTDSDANASIGMKKYFVGINAHGHVAISAKKIEGENIGAQVLG
LFTLSSGKDIWQKLS

SEQ ID NO: 9 (pCART201)
SVWTKGVTPLTPEQFRYNNEDVIHAPYLAHQGWYDITKAFDGKDNLLCGAATAGNMLHWWFDQNKTEIEAYLSKH
PEKQKIIFNNQELFDLKAAIDTKDSQTNSQLFNYFRDKAFPNLSARQLGVMPDLVLDMFINGYYLNVFKTQSTDV
NRPYQDKDKRGGIFDAVFTRGDQTTLLTARHDLKNKGLNDISTIIKQELTEGRALALSHTYANVSISHVINLWGA
DFNAEGNLEAIYVTDSDANASIGMKKYFVGINAHGHVAISAKKIEGENIGAQVLGLFTLSSGKDIWQKLS

SEQ ID NO: 10 (pCART202)
DDYQRNATEAYAKEVPHQITSVWTKGVTPLTPEQFTQGEDVIHAPYLAHQGWYDITKAFDGKDNLLCGAATAGNM
LHWWFDQNKTEIEAYLSKHPEKQKIIFNNQELFDLKAAIDTKDSQTNSQLFNYFRDKAFPNLSARQLGVMPDLVL
DMFINGYYLNVFKTQSTDVNRPYQDKDKRGGIFDAVFTRGDQTTLLTARHDLKNKGLNDISTIIKQELTEGRALA
LSHTYANVSISHVINLWGADFNAEGNLEAIYVTDSDANASIGMKKYFVGINAHGHVAISAKKIEGENIGAQVLGL
FTLSSGKDIWQKLS

SEQ ID NO: 11 (pCART203)
DDYQRNATEAYAKEVPHQITSVWTKGVTPPEQFTQGEDVIHAPYLAHQGWYDITKAFDGKDNLLCGAATAGNMLH
WWFDQNKTEIEAYLSKHPEKQKIIFNNQELFDLKAAIDTKDSQTNSQLFNYFRDKAFPNLSARQLGVMPDLVLDM
FINGYYLNVFKTQSTDVNRPYQDKDKRGGIFDAVFTRGDQTTLLTARHDLKNKGLNDISTIIKQELTEGRALALS
HTYANVSISHVINLWGADFNAEGNLEAIYVTDSDANASIGMKKYFVGINAHGHVAISAKKIEGENIGAQVLGLFT
LSSGKDIWQKLS

SEQ ID NO: 12 (pCART204)
DDYQRNATEAYAKEVPHQITSVWTKGVTPPEQFRYNNEDVIHAPYLAHQGWYDITKAFDGKDNLLCGAATAGNML
HWWFDQNKTEIEAYLSKHPEKQKIIFNNQELFDLKAAIDTKDSQTNSQLFNYFRDKAFPNLSARQLGVMPDLVLD
MFINGYYLNVFKTQSTDVNRPYQDKDKRGGIFDAVFTRGDQTTLLTARHDLKNKGLNDISTIIKQELTEGRALAL
SHTYANVSISHVINLWGADFNAEGNLEAIYVTDSDANASIGMKKYFVGINAHGHVAISAKKIEGENIGAQVLGLF
TLSSGKDIWQKLS

SEQ ID NO: 13 (pCART 206)
DDYQRNATEAYAKEVPHQITSVWTKGVTPPEQFTQGEDVIHAPYLAHQGWYDITKAFDGKDNLLCGAATAGNMLH
WWFDQNKTEIEAYLSKHPEKQKIIINNQELFDLKAAIDTKDSQTNSQLFNYFRDKAFPNLSARQLGVMPDLVLDM
FINGYYLNVFKTQSTDVNRPYQDKDKRGGIFDAVFTRGDQTTLLTARHDLKNKGLNDISTIIKQELTEGRALALS
HTYANVSISHVINLWGADFNAEGNLEAIYVTDSDANASIGMKKYFVGINAHGHVAISAKKIEGENIGAQVLGLFT
LSSGKDIWQKLS

SEQ ID NO: 14 (pCART207)
DDYQRNATEAYAKEVPHQITSVWTKGVTPPEQFTQGEDVIHAPYLAHQGWYDITKAFDGKDNLLCGAATAGNMLH
WWFDQNKTEIEAYLSKHPEKQKIIFRNQELFDLKAAIDTKDSQTNSQLFNYFRDKAFPNLSARQLGVMPDLVLDM
FINGYYLNVFKTQSTDVNRPYQDKDKRGGIFDAVFTRGDQTTLLTARHDLKNKGLNDISTIIKQELTEGRALALS
HTYANVSISHVINLWGADFNAEGNLEAIYVTDSDANASIGMKKYFVGINAHGHVAISAKKIEGENIGAQVLGLFT
LSSGKDIWQKLS

SEQ ID NO: 15 (pCART208)
DDYQRNATEAYAKEVPHQITSVWTKGVTPPEQFTQGEDVIHAPYLAHQGWYDITKAFDGKDNLLCGAATAGNMLH
WWFDQNKTEIEAYLSKHPEKQKIIIRNQELFDLKAAIDTKDSQTNSQLFNYFRDKAFPNLSARQLGVMPDLVLDM
FINGYYLNVFKTQSTDVNRPYQDKDKRGGIFDAVFTRGDQTTLLTARHDLKNKGLNDISTIIKQELTEGRALALS
HTYANVSISHVINLWGADFNAEGNLEAIYVTDSDANASIGMKKYFVGINAHGHVAISAKKIEGENIGAQVLGLFT
LSSGKDIWQKLS

SEQ ID NO: 16 (pCART210)
DDYQRNATEAYAKEVPHQITSVWTKGVTPPEQFTQGEDVIHAPYLANQGWYDITKAFDGKDNLLCGAATAGNMLH
WWFDQNKTEIEAYLSKHPEKQKIIFRNQELFDLKEAIRTKDSQTNSQLFEYFRDKAFPYLSARQLGVMPDLVLDM
FINGYYLNVFKTQSTDVKRPYQDKDKRGGIFDAVFTRGNQTTLLTARHDLKNKGLNDISTIIKEELTKGRALALS
HTYANVSISHVINLWGADFNAEGNLEAIYVTDSDANASIGMKKYFVGINKHGHVAISAKKIEGENIGAQVLGLFT
LSSGKDIWQKLN

SEQ ID NO: 17 (pCART217)
SVWTKGVTPPEQFTQGEDVIHAPYLAHQGWYDITKAFDGKDNLLCGAATAGNMLHWWFDQNKTEIEAYLSKHPEK
QKIIFRNQELFDLKAAIDTKDSQTNSQLFNYFRDKAFPNLSARQLGVMPDLVLDMFINGYYLNVFKTQSTDVNRP

YQDKDKRGGIFDAVFTRGNQTTLLTARHDLKNKGLNDISTIIKQELTEGRALALSHTYANVSISHVINLWGADFN
AEGNLEAIYVTDSDANASIGMKKYFVGINAHGHVAISAKKIEGENIGAQVLGLFTLSSGKDIWQKLS

SEQ ID NO: 18 (pCART219)
DDYQRNATEAYAKEVPHQITSVWTKGVTPPEQFTQGEDVIHAPYLAHQGWYDITKAFDG**A**DNLLCGAATAGNMLH
WWFDQNKTEIEAYLSKHPEKQKIIFR**N**QELFDLKAAIDTKDSQTNSQLFNYFRDKAFPNLSARQLGVMPDLVLDM
FINGYYLNVFKTQSTDVNRPYQDKDKRGGIFDAVFTRG**N**QTTLLTARHDLKNKGLNDISTIIKQELTEGRALALS
HTYANVSISHVINLWGADFNAEGNLEAIYVTDSDANASIGMKKYFVGINAHGHVAISAKKIEGENIGAQVLGLFT
LSSGKDIWQKLS

SEQ ID NO: 19 (pCART226)
SVWTKGVTPPEQFTQGEDVIHAPYLAHQGWYDITKAFDGADNLLCGAATAGNMLHWWFDQNKTEIEAYLSKHPEK
QKIIFRNQELFDLKAAIDTKDSQTNSQLFNYFRDKAFPNLSARQLGVMPDLVLDMFINGYYLNVFKTQSTDVNRP
YQDKDKRGGIFDAVFTRGNQTTLLTARHDLKNKGLNDISTIIKQELTEGRALALSHTYANVSISHVINLWGADFN
AEGNLEAIYVTDSDANASIGMKKYFVGINAHGHVAISAKKIEGENIGAQVLGLFTLSSGKDIWQKLS

SEQ ID NO: 20 (pCART229)
DDYQRNATEAYAKEVPHQITSVWTKGVTPPEQFTQGEDVIHAPYLAHQGWYDITKAFDGKDNLLCGAATAGNMLH
WWFDQNKTEIEAYLSKHPEKQKIIFRNQELFDLKAAIDTKDSQTNSQLFNYFRDKAFPNLSARQLGVMPDLVLDM
FINGYYLNVFKTQSTDVNRPYQDKDKRGGIFDAVFTRGNQTTLLTARHDLKNKGLNDISTIIKQELTEGRALALS
HTYANVSISHVINLWGADFNAEGNLEAIYVTDSDANASIGMKKYFVGINAHGHVAISAKKIEGENIGAQVLGLFT
LSSGKDIWQKLS

SEQ ID NO: 21 (pCART191)
DDYQRNATEAYAKEVPHQITSVWTKGVTPLTPEQFTQGEDVFHAPYVANQGWYDITKAFDGKDNLLCGAATAGNM
LHWWFDQNKDQIKRYLEEHPEKQKINFNGENMFDVKKAIDTKNHQLDSKLFNYFKEKAFPYLSAKHLGVFPDHVI
DMFINGYRLSLTNHGPTPVKEGSKDPRGGIFDAVFTRGNQSKLLTSRHDFKNKNLNDISTIIKQELTKGKALGLS
HTYANVRINHVINLWGADFNAEGNLEAIYVTDSDSNASIGMKKYFVGVNAHGHVAISAKKIEGENIGAQVLGLFT
LSTGQDSWQKLS

SEQ ID NO: 22 (pCART192)
DDYQRNATEAYAKEVPHQITSVWTKGVTPLTPEQFTQGEDVFHAPYVANQGWYDITKAFDGKDNLLCGAATAGNM
LHWWFDQNKDQIKRYLEEHPEKQKINFRGENMFDVKEAIRTKNHQLDSKLFEYFKEKAFPYLSAKHLGVFPDHVI
DMFINGYRLSLTNHGPTPVKKGSKDPRGGIFDAVFTRGNQSKLLTSRHDFKNKNLNDISTIIKSELTNGKALGLS
HTYANVRINHVINLWGADFNAEGNLEAIYVTDSDSNASIGMKKYFVGVNKHGHVAISAKKIEGENIGAQVLGLFT
LSTGQDSWQKLN

SEQ ID NO: 23 (pCART193)
DDYQRNATEAYAKEVPHQITSVWTKGVTPLTPEQFTQGEDVFHAPYVANQGWYDITKTFNGKDDLLCGAATAGNM
LHWWFDQNKDQIKRYLEEHPEKQKINFNGEQMFDVKEAIDTKNHQLDSKLFEYFKEKAFPYLSTKHLGVFPDHVI
DMFINGYRLSLTNHGPTPVKEGSKDPRGGIFDAVFTRGNQSKLLTSRHDFKEKNLKEISDLIKQELTEGKALGLS
HTYANVRINHVINLWGADFDAEGNLKAIYVTDSDSNASIGMKKYFVGVNAAGKVAISAKKIEGENIGAQVLGLFT
LSTGQDSWNQTS

SEQ ID NO: 24 (pCART194)
DDYQRNATEAYAKEVPHQITSVWTKGVTPLTPEQFTQGEDVFHAPYVANQGWYDITKTFNGKDDLLCGAATAGNM
LHWWFDQNKDQIKRYLEEHPEKQKINFNGEQMFDVKEAIRTKNHQLDSKLFEYFKEKAFPYLSTKHLGVFPDHVI
DMFINGYRLSLTNHGPTPVKKGSKDPRGGIFDAVFTRGNQSKLLTSRHDFKEKNLKEISDLIKEELTKGKALGLS
HTYANVRINHVINLWGADFDAEGNLKAIYVTDSDSNASIGMKKYFVGVNKAGKVAISAKKIEGENIGAQVLGLFT
LSTGQDSWNQTN

SEQ ID NO: 25 (pCART205)
DDYQRNATEAYAKEVPHQITSVWTKGVTPPEQFTQGEDVIHAPYVANQGWYDITKAFDGKDNLLCGAATAGNMLH
WWFDQNKDQIKRYLEEHPEKQKINFNGEQMFDVKKAIDTKNHQLDSKLFNYFKEKAFPGLSARRIGVFPDHVIDM
FINGYRLSLTNHGPTPVKEGSKDPRGGIFDAVFTRGNQSKLLTSRHDFKNKNLNDISTIIKQELTKGKALGLSHT
YANVSINHVINLWGADFNAEGNLEAIYVTDSDSNASIGMKKYFVGVNAHGHVAISAKKIEGENIGAQVLGLFTLS
TGQDSWQKLS

The polypeptide of the invention may comprise, consist essentially, or consist of the sequence of any one of SEQ ID NOs: 6 to 25. Each of SEQ ID NOs: 6 to 25 may optionally include an

additional methionine at the N terminus and/or a histidine tag at the C terminus. The histidine tag is preferably consists of six histidine residues. The histidine tag is preferably linked to the C terminus by a linker of 3x glycine or 5x glycine residues.

Production of polypeptides

A polypeptide as disclosed herein may be produced by any suitable means. For example, the polypeptide may be synthesised directly using standard techniques known in the art, such as Fmoc solid phase chemistry, Boc solid phase chemistry or by solution phase peptide synthesis. Alternatively, a polypeptide may be produced by transforming a cell, typically a bacterial cell, with a nucleic acid molecule or vector which encodes said polypeptide. Production of polypeptides by expression in bacterial host cells is described below and is exemplified in the Examples. The invention provides nucleic acid molecules and vectors which encode a polypeptide of the invention. The invention also provides a host cell comprising such a nucleic acid or vector. Exemplary polynucleotide molecules encoding polypeptides disclosed herein are provided as SEQ ID NOs: 33 to 55. Each of these sequences includes at the 3' end a codon for the N terminal methionine (ATG) and, prior to the stop codon (TAA) at the 5' end, codons for a 3x gly linker and a 6x his histidine tag, which may optionally be excluded.

The terms "nucleic acid molecule" and "polynucleotide" are used interchangeably herein and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Non-limiting examples of polynucleotides include a gene, a gene fragment, messenger RNA (mRNA), cDNA, recombinant polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide of the invention may be provided in isolated or substantially isolated form. By substantially isolated, it is meant that there may be substantial, but not total, isolation of the polypeptide from any surrounding medium. The polynucleotides may be mixed with carriers or diluents which will not interfere with their intended use and still be regarded as substantially isolated. A nucleic acid sequence which "encodes" a selected polypeptide is a nucleic acid molecule which is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences, for example in an expression vector. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. For the purposes of the invention, such nucleic acid sequences can include, but are not limited to, cDNA from viral, prokaryotic

or eukaryotic mRNA, genomic sequences from viral or prokaryotic DNA or RNA, and even synthetic DNA sequences. A transcription termination sequence may be located 3' to the coding sequence.

Polynucleotides can be synthesised according to methods well known in the art, as described by way of example in Sambrook *et al* (1989, Molecular Cloning - a laboratory manual; Cold Spring Harbor Press). The nucleic acid molecules of the present invention may be provided in the form of an expression cassette which includes control sequences operably linked to the inserted sequence, thus allowing for expression of the polypeptide of the invention *in vivo*. These expression cassettes, in turn, are typically provided within vectors (e.g., plasmids or recombinant viral vectors). Such an expression cassette may be administered directly to a host subject. Alternatively, a vector comprising a polynucleotide of the invention may be administered to a host subject. Preferably the polynucleotide is prepared and/or administered using a genetic vector. A suitable vector may be any vector which is capable of carrying a sufficient amount of genetic information, and allowing expression of a polypeptide of the invention.

The present invention thus includes expression vectors that comprise such polynucleotide sequences. Such expression vectors are routinely constructed in the art of molecular biology and may for example involve the use of plasmid DNA and appropriate initiators, promoters, enhancers and other elements, such as for example polyadenylation signals which may be necessary, and which are positioned in the correct orientation, in order to allow for expression of a peptide of the invention. Other suitable vectors would be apparent to persons skilled in the art. By way of further example in this regard we refer to Sambrook *et al*.

The invention also includes cells that have been modified to express a polypeptide of the invention. Such cells typically include prokaryotic cells such as bacterial cells, for example *E. coli*. Such cells may be cultured using routine methods to produce a polypeptide of the invention.

A polypeptide may be derivatised or modified to assist with their production, isolation or purification. For example, where a polypeptide of the invention is produced by recombinant expression in a bacterial host cell, the sequence of the polypeptide may include an additional methionine (M) residue at the N terminus to improve expression. As another example, the polypeptide of the invention may be derivatised or modified by addition of a ligand which is capable of binding directly and specifically to a separation means. Alternatively, the polypeptide may be derivatised or modified by addition of one member of a

binding pair and the separation means comprises a reagent that is derivatised or modified by addition of the other member of a binding pair. Any suitable binding pair can be used. In a preferred embodiment where the polypeptide for use in the invention is derivatised or modified by addition of one member of a binding pair, the polypeptide is preferably histidine-tagged or biotin-tagged. Typically the amino acid coding sequence of the histidine or biotin tag is included at the gene level and the polypeptide is expressed recombinantly in *E. coli*. The histidine or biotin tag is typically present at either end of the polypeptide, preferably at the C-terminus. It may be joined directly to the polypeptide or joined indirectly by any suitable linker sequence, such as 3, 4 or 5 glycine residues. The histidine tag typically consists of six histidine residues, although it can be longer than this, typically up to 7, 8, 9, 10 or 20 amino acids or shorter, for example 5, 4, 3, 2 or 1 amino acids.

The amino acid sequence of a polypeptide may be modified to include non-naturally occurring amino acids, for example to increase stability. When the polypeptides are produced by synthetic means, such amino acids may be introduced during production. The polypeptides may also be modified following either synthetic or recombinant production. Polypeptides may also be produced using D-amino acids. In such cases the amino acids will be linked in reverse sequence in the C to N orientation. This is conventional in the art for producing such polypeptides.

A number of side chain modifications are known in the art and may be made to the side chains of the polypeptides, subject to the polypeptides retaining any further required activity or characteristic as may be specified herein. It will also be understood that polypeptides may be chemically modified, e.g. post-translationally modified. For example, they may be glycosylated, phosphorylated or comprise modified amino acid residues.

The polypeptide may be PEGylated. The polypeptide of the invention may be in a substantially isolated form. It may be mixed with carriers or diluents (as discussed below) which will not interfere with the intended use and still be regarded as substantially isolated. It may also be in a substantially purified form, in which case it will generally comprise at least 90%, e.g. at least 95%, 98% or 99%, of the protein in the preparation.

Compositions and formulations comprising polypeptides

In another aspect, the present invention provides compositions comprising a polypeptide of the invention. For example, the invention provides a composition comprising one or more polypeptides of the invention, and at least one pharmaceutically acceptable carrier or diluent. The carrier (s) must be 'acceptable' in the sense of being compatible with

the other ingredients of the composition and not deleterious to a subject to which the composition is administered. Typically, carriers and the final composition, are sterile and pyrogen free.

Formulation of a suitable composition can be carried out using standard pharmaceutical formulation chemistries and methodologies all of which are readily available to the reasonably skilled artisan. For example, the agent can be combined with one or more pharmaceutically acceptable excipients or vehicles. Auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, reducing agents and the like, may be present in the excipient or vehicle. Suitable reducing agents include cysteine, thioglycerol, thioreducin, glutathione and the like. Excipients, vehicles and auxiliary substances are generally pharmaceutical agents that do not induce an immune response in the individual receiving the composition, and which may be administered without undue toxicity. Pharmaceutically acceptable excipients include, but are not limited to, liquids such as water, saline, polyethyleneglycol, hyaluronic acid, glycerol, thioglycerol and ethanol. Pharmaceutically acceptable salts can also be included therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients, vehicles and auxiliary substances is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

Such compositions may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable compositions may be prepared, packaged, or sold in unit dosage form, such as in ampoules or in multi-dose containers containing a preservative. Compositions include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such compositions may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a composition for parenteral administration, the active ingredient is provided in dry (for e.g., a powder or granules) form for reconstitution with a suitable vehicle (e. g., sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition. The compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally-

acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono-or di-glycerides.

Other parentally-administrable compositions which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer systems. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt. The compositions may be suitable for administration by any suitable route including, for example, intradermal, subcutaneous, percutaneous, intramuscular, intra-arterial, intraperitoneal, intraarticular, intraosseous or other appropriate administration routes. Preferred compositions are suitable for administration by intravenous infusion.

Methods of use of polypeptides

The invention provides for the use of polypeptides of the invention in various methods. For example, the present polypeptides may provide useful tools for biotechnology. The polypeptides may be used for specific *ex vivo* cleavage of IgG, in particular human IgG. In such a method, the polypeptide may be incubated with a sample containing IgG under conditions which permit the specific cysteine protease activity to occur. Specific cleavage can be verified, and the cleavage products isolated using any suitable method, such as those described in WO2003051914 and WO2009033670. Thus the method can be used in particular to generate Fc and $F(ab')_2$ fragments. Fab fragments may then be produced by carrying out a reduction step (for example in 2-mercaptoethanolamine or Cysteamine) on the $F(ab')_2$ fragments that result from cleavage of IgG with a polypeptide of the invention.

The method may also be used to detect or analyse IgG in a sample, or to remove IgG from a sample. A method for the detection of IgG in a sample typically involves incubating the polypeptide with the sample under conditions which permit IgG-specific binding and cleavage. The presence of IgG can be verified by detection of the specific IgG cleavage products, which may subsequently be analysed.

The polypeptides in accordance with the present invention may also be used in therapy or prophylaxis. In therapeutic applications, polypeptides or compositions are administered to a subject already suffering from a disorder or condition, in an amount sufficient to cure, alleviate or partially arrest the condition or one or more of its symptoms. Such therapeutic treatment may result in a decrease in severity of disease symptoms, or an

increase in frequency or duration of symptom-free periods. An amount adequate to accomplish this is defined as "therapeutically effective amount". In prophylactic applications, polypeptides or compositions are administered to a subject not yet exhibiting symptoms of a disorder or condition, in an amount sufficient to prevent or delay the development of symptoms. Such an amount is defined as a "prophylactically effective amount". The subject may have been identified as being at risk of developing the disease or condition by any suitable means. Thus the invention also provides a polypeptide of the invention for use in the treatment of the human or animal body. Also provided herein is a method of prevention or treatment of disease or condition in a subject, which method comprises administering a polypeptide of the invention to the subject in a prophylactically or therapeutically effective amount. The polypeptide may be co-administered with an immune-suppressive agent. The polypeptide is preferably administered by intravenous infusion, but may be administered by any suitable route including, for example, intradermal, subcutaneous, percutaneous, intramuscular, intra-arterial, intraperitoneal, intraarticular, intraosseous or other appropriate administration routes. The amount of said polypeptide that is administered may be between 0.01mg/kg BW and 2mg/kg BW, between 0.04 and 2mg/kg BW, between 0.12mg/kg BW and 2mg/kg BW, preferably between 0.24mg/kg and 2mg/kg BW and most preferably between 1mg/kg and 2mg/kg BW. The polypeptide may be administered on multiple occasions to the same subject, provided that the quantity of ADA in the serum of the subject which is capable of binding to the polypeptide does not exceed a threshold determined by the clinician. The quantity of ADA in the serum of the subject which is capable of binding to the polypeptide may be determined by any suitable method, such as an agent specific CAP FEIA (ImmunoCAP) test or a titre assay.

Polypeptides of the invention may be particularly useful in the treatment or prevention of a disease or condition mediated by pathogenic IgG antibodies. Accordingly, the invention provides a polypeptide of the invention for use in the treatment or prevention of a disease or condition mediated by pathogenic IgG antibodies. The invention also provides a method of treating or preventing a disease or condition mediated by pathogenic IgG antibodies comprising administering to an individual a polypeptide of the invention. The method may comprise repeat administration of the said polypeptide. The invention also provides a polypeptide of the invention for use in the manufacture of a medicament for the treatment or prevention of a disease or condition mediated by pathogenic IgG antibodies, particularly an autoimmune disease which is mediated in whole or in part by pathogenic IgG antibodies.

The pathogenic antibodies may typically be specific for an antigen which is targeted in an autoimmune disease or other condition mediated wholly or in part by antibodies. Table D sets out a list of such diseases and the associated antigens. A polypeptide of the invention may be used to treat any of these diseases or conditions. The polypeptide is particularly effective for the treatment or prevention of autoimmune disease which is mediated in whole or in part by pathogenic IgG antibodies.

Table D

DISEASE	**AUTOANTIGENS**
Addison's disease	Steroid 21-hydroxylase, 17 alpha-Hydroxylase (17OH) and side-chain-cleavage enzyme (P450scc), Thyroperoxidase, thyroglobulin and H+/K(+)-
Anti-GBM glomerulonephritis (related to Goodpasteur)	Anti-glomerular basement membrane (anti-GBM): noncollagenous (NC1) domains of the alpha3alpha4alpha5(IV) collagen
Anti-neutrophil cytoplasmic antibody-associated vasculitides (ANCA associated vasculitis)(Wegener granulomatosis, Churg-Strauss syndrome, microscopic polyangiitis)	Myeloperoxidase, proteinase 3
Anti-NMDAR Encephalitis	N-methyl-D-aspartate receptor (NMDAR)
Anti-phospholipid antibody syndrome (APS) and catastrophic APS	Negatively-charged phospholipids complexed with phospholipid binding plasma proteins (e.g. beta2GPI), cardiolipin, beta2-glycoprotein I, and (beta2GPI)
Autoimmune bullous skin diseases (Pemphigus). Pemphigus foliaceus (PF), fogo selvagem (FS)(endemic form), pemphigus vulgaris (PV)	IgG against keratinocytes. Specific target is desmoglein (Dsg) 1 (desmosomal Cadherins)
Autoimmune hemolytic anemia (AIHA)	Self-antigens on red-blood-cells
Autoimmune hepatitis (AIH)	Actin, antinuclear antibody (ANA), smooth muscle antibody (SMA), liver/kidney microsomal antibody (LKM-1), anti soluble liver antigen (SLA/LP) and anti-mitochondrial antibody (AMA), CYP2D6, CYP2C9-tienilic acid, UGT1A, CYP1A2, CYP2A6, CYP3A, CYP2E1, CYP11A1, CYP17 and CYP21
Autoimmune neutropenia (AIN)	FcgRIIIb
Bullous pemphigoid (BP)	Hemidesmosomal proteins BP230 and BP180 (type XVII collagen), laminin 5, the alpha6 subunit of the integrin alpha6beta4 and p200
Celiac disease	transglutaminase 2 (TG2), transglutaminase 3, actin, ganglioside, collagen, calreticulin and zonulin, thyroid, endocrine pancreas, anti-gastric and liver, anti-nuclear constituents, anti-reticulin, actin, smooth muscle, calreticulin, desmin, collagens, bone, anti-brain, ganglioside, neuronal, blood vessel
Chronic utricaria	Alpha-subunit of the high-affinity IgE receptor, IgE

Complete congenital heart block (CCHB)	Ro (Sjögens syndrome antigen A (SSA)), La (Sjögens syndrome antigen B(SSB))
Diabetes type 1A (T1DM)	Islet cell autoantibodies (ICA), antibodies to insulin (IAA), glutamic acid decarboxylase (GAA or GAD), protein tyrosine phosphatase (IA2 or ICA512), Insulinoma Associated Peptide-2. The number of antibodies, rather than the individual antibody, is thought to be most predictive of progression to overt diabetes.
Epidermolysis bullosa acquisita (EBA)	The 145-kDa noncollagenous aminoterminal (NC-1) domain of collagen VII
Essential mixed cryoglobulinemia	Essential mixed cryoglobulinemia antigens
Goodpasture´s syndrome (also known as Goodpasture's disease and anti-glomerular basement membrane disease	alpha3(IV) collagen (=Goodpasture antigen)
Graves´disease (Basedow´s disease), includes Goitre and hyperthyroidism, infiltrative exopthalmos and infiltarative dermopathy.	Thyrotropin receptor (TSHR) Thyroid peroxidase (TPO)
Guillain-Barré syndrome (GBS). Acute inflammatory demyelinating polyneuropathy (AIDP), acute motor axonal neuropathy (AMAN)	Gangliosides GM1, GM1b, GD1a, and GalNAc-GD1a, glycosphingolipid, myelin proteins PMP22 and P0
Hemophilia - Acquired FVIII deficiency	Factor VIII
Idiopathic thrombocytopenic purpura (ITP)	Platelet glycoprotein (GP) IIb-IIIa and/or GPIb-IX
Lambert-Eaton myasthenic syndrome (LEMS)	voltage gated calcium channels
Mixed Connective Tissue Disease (MCTD)	IgG directed against the spliceosome, U1-snRNP
Multiple Myeloma	Multiple Myeloma antigens
Myasthenia gravis Myasthenic crisis	Acetylcholine receptors (AchR), muscle-specific kinase (MuSK)
Myocarditis, dilated cardiomyopathy (DCM)(congestive cardiomyopathy)	heart-reactive autoantibodies against multiple antigens e.g. cardiac myosin
Neuromyelitis Optica (NMO)	Aquaporin 4 (AQP4)
Primary biliary cirrhosis (PBC)	pyruvate dehydrogenase complex (PDC)-E2 and other members of the oxaloacid dehydrogenase family, Glycoprotein-210, p62, sp100
Primary Progressive Multiple Sclerosis (PPMS)	Myelin oligodendrocyte glycoprotein (MOG),Myelin proteolipid protein (PLP), transketolase (TK), cyclic nucleotide phosphodiesterase type I (CNPase I), collapsin response mediator protein 2, tubulin beta4, neurofascin
Rheumatic heart disease (RHD),(Rheumatic fever)	Cardiac myosin

Rheumatoid Arthritis (RA)	Type II collagen, citrullin (-ated proteins (e.g. (fibrinogen, vimentin, filaggrin, type II collagen, enolase)), G6PI, RFs (anti-Fc/IgG), Vimentin, and cytokeratin
Serum-sickness, immune complex hypersensitivity (type III)	Various antigens
Sjögren Syndrome (SS)	Ro (Sjögens syndrome antigen A (SS-A)), La (Sjögens syndrome antigen B(SS-B)), p80 coilin, antinuclear antibodies, anti-thyroid, anti-centromere antibodies (Raynaud's phenomenon), anti-carbonic anhydrase II (distal renal tubular acidosis), anti-mitochondrial antibodies (liver pathology), cryoglobulins (evolution to non-Hodgkin's lymphoma). alpha- and beta-fodrin, islet cell autoantigen, poly(ADP)ribose polymerase (PARP), NuMA, Golgins, NOR-90, M3-muscarinic receptor
SLE including Lupus nephritis	Autoantibodies to nuclear constituents (e.g. dsDNA and nucleosomes), dsDNA, PARP, Sm, PCDA, rRNA Ribosome P proteins, C1q
Stiff-person syndrome (SPS)	glutamic acid decarboxylase (GAD), amphiphysin.
Systemic sclerosis (scleroderma)	DNA-topoisomerase I (Scl-70), U3 snRNP, U2 snRNP, 7-2 RNP, NOR-90, centromere-associated proteins, and nucleolar antigens ,Anti-Th/To, Anti-RNA polymerase I/III, Anti-PDGF receptor, Anti-fibrillin-1, M3-muscarinic receptor,
Transplant rejection	Transplant rejection antigens
Thrombotic Thrombocytopenic Purpura (TTP)	ADAMTS13

In another embodiment, a polypeptide of the invention may be used in a method to improve the benefit to a subject of a therapy or a therapeutic agent. The method comprises two steps, which are referred to herein as steps (a) and (b).

Step (a) comprises administering to the subject a polypeptide of the invention. The amount of the polypeptide administered is preferably sufficient to cleave substantially all IgG molecules present in the plasma of the subject. Step (b) comprises subsequently administering to the subject the said therapy or therapeutic agent. Steps (a) and (b) are separated by a time interval which is preferably sufficient for cleavage of substantially all IgG molecules present in the plasma of the subject to take place. The said interval may typically be of at least 30 minutes and at most 21 days.

The therapeutic agent of which the benefit is improved is typically an antibody which is administered for the treatment of cancer or another disease. The therapeutic agent may be IVIg. In the context of this embodiment, the invention may be alternatively described as providing a method for the treatment of cancer or another disease in a subject, the method comprising (a) administering to the subject a polypeptide of the invention; and (b)

subsequently administering to the subject a therapeutically effective amount of an antibody which is a treatment for said cancer or said other disease; wherein:

- the amount of said polypeptide administered is sufficient to cleave substantially all IgG molecules present in the plasma of the subject; and
- steps (a) and (b) are separated by a time interval of at least 2 hours and at most 21 days.

In other words, the invention also provides the polypeptide for use in such a method for the treatment of cancer or another disease. The invention also provides use of the agent in the manufacture of a medicament for the treatment of cancer or another disease by such a method. The cancer may be Acute lymphoblastic leukemia, Acute myeloid leukemia, Adrenocortical carcinoma, AIDS-related cancers, AIDS-related lymphoma, Anal cancer, Appendix cancer, Astrocytoma, childhood cerebellar or cerebral, Basal cell carcinoma, Bile duct cancer, extrahepatic, Bladder cancer, Bone cancer, Osteosarcoma/Malignant fibrous histiocytoma, Brainstem glioma, Brain cancer, Brain tumor, cerebellar astrocytoma, Brain tumor, cerebral astrocytoma/malignant glioma, Brain tumor, ependymoma, Brain tumor, medulloblastoma, Brain tumor, supratentorial primitive neuroectodermal tumors, Brain tumor, visual pathway and hypothalamic glioma, Breast cancer, Bronchial adenomas/carcinoids, Burkitt lymphoma, Carcinoid tumor, Carcinoid tumor, gastrointestinal, Carcinoma of unknown primary, Central nervous system lymphoma, Cerebellar astrocytoma, Cerebral astrocytoma/Malignant glioma, Cervical cancer, Chronic lymphocytic leukemia, Chronic myelogenous leukemia Chronic myeloproliferative disorders, Colon Cancer, Cutaneous T-cell lymphoma, Desmoplastic small round cell tumor, Endometrial cancer, Ependymoma, Esophageal cancer, Ewing's sarcoma in the Ewing family of tumors, Extracranial germ cell tumor, Childhood, Extragonadal Germ cell tumor, Extrahepatic bile duct cancer, Eye Cancer, Intraocular melanoma, Eye Cancer, Retinoblastoma, Gallbladder cancer, Gastric (Stomach) cancer, Gastrointestinal Carcinoid Tumor, Gastrointestinal stromal tumor (GIST), Germ cell tumor: extracranial, extragonadal, or ovarian, Gestational trophoblastic tumor, Glioma of the brain stem, Glioma, Childhood Cerebral Astrocytoma, Glioma, Childhood Visual Pathway and Hypothalamic, Gastric carcinoid, Hairy cell leukemia, Head and neck cancer, Heart cancer, Hepatocellular (liver) cancer, Hodgkin lymphoma, Hypopharyngeal cancer, Hypothalamic and visual pathway glioma, Intraocular Melanoma, Islet Cell Carcinoma (Endocrine Pancreas), Kaposi sarcoma, Kidney cancer (renal cell cancer), Laryngeal Cancer, Leukemias, Leukemia, acute lymphoblastic (also called acute lymphocytic leukemia), Leukemia, acute myeloid (also called acute myelogenous

leukemia), Leukemia, chronic lymphocytic (also called chronic lymphocytic leukemia), Leukemia, chronic myelogenous (also called chronic myeloid leukemia), Leukemia, hairy cell, Lip and Oral Cavity Cancer, Liposarcoma, Liver Cancer (Primary), Lung Cancer, Non-Small Cell Lung Cancer, Small Cell, Lymphomas, Lymphoma, AIDS-related, Lymphoma, Burkitt, Lymphoma, cutaneous T-Cell, Lymphoma, Hodgkin, Lymphomas, Non-Hodgkin (an old classification of all lymphomas except Hodgkin's), Lymphoma, Primary Central Nervous System, Macroglobulinemia, Waldenström, Malignant Fibrous Histiocytoma of Bone/Osteosarcoma, Medulloblastoma, Melanoma, Melanoma, Intraocular (Eye), Merkel Cell Carcinoma, Mesothelioma, Adult Malignant, Mesothelioma, Metastatic Squamous Neck Cancer with Occult Primary, Mouth Cancer, Multiple Endocrine Neoplasia Syndrome, Multiple Myeloma/Plasma Cell Neoplasm, Mycosis Fungoides, Myelodysplastic Syndromes, Myelodysplastic/Myeloproliferative Diseases, Myelogenous Leukemia, Chronic, Myeloid Leukemia, Adult Acute, Myeloid Leukemia, Childhood Acute, Myeloma, Multiple (Cancer of the Bone-Marrow), Myeloproliferative Disorders, Nasal cavity and paranasal sinus cancer, Nasopharyngeal carcinoma, Neuroblastoma, Non-Hodgkin lymphoma, Non-small cell lung cancer, Oral Cancer, Oropharyngeal cancer, Osteosarcoma/malignant fibrous histiocytoma of bone, Ovarian cancer, Ovarian epithelial cancer (Surface epithelial-stromal tumor), Ovarian germ cell tumor, Ovarian low malignant potential tumor, Pancreatic cancer, Pancreatic cancer, islet cell, Paranasal sinus and nasal cavity cancer, Parathyroid cancer, Penile cancer, Pharyngeal cancer, Pheochromocytoma, Pineal astrocytoma, Pineal germinoma, Pineoblastoma and supratentorial primitive neuroectodermal tumors, Pituitary adenoma, Plasma cell neoplasia/Multiple myeloma, Pleuropulmonary blastoma, Primary central nervous system lymphoma, Prostate cancer, Rectal cancer, Renal cell carcinoma (kidney cancer), Renal pelvis and ureter, transitional cell cancer, Retinoblastoma, Rhabdomyosarcoma, Salivary gland cancer, Sarcoma, Ewing family of tumors, Kaposi Sarcoma, Sarcoma, soft tissue, Sarcoma, uterine, Sézary syndrome, Skin cancer (nonmelanoma), Skin cancer (melanoma), Skin carcinoma, Merkel cell, Small cell lung cancer, Small intestine cancer, Soft tissue sarcoma, Squamous cell carcinoma, Squamous neck cancer with occult primary, metastatic, Stomach cancer, Supratentorial primitive neuroectodermal tumor, T-Cell lymphoma, cutaneous – see Mycosis Fungoides and Sézary syndrome, Testicular cancer, Throat cancer, Thymoma, Thymoma and Thymic carcinoma, Thyroid cancer, Thyroid cancer, Transitional cell cancer of the renal pelvis and ureter, Trophoblastic tumor, Ureter and renal pelvis, transitional cell cancer Urethral cancer, Uterine

cancer, endometrial, Uterine sarcoma, Vaginal cancer, Visual pathway and hypothalamic glioma, Vulvar cancer, Waldenström macroglobulinemia and Wilms tumor (kidney cancer).

The cancer is preferably prostate cancer, breast cancer, bladder cancer, colon cancer, rectal cancer, pancreatic cancer, ovarian cancer, lung cancer, cervical cancer, endometrial cancer, kidney (renal cell) cancer, oesophageal cancer, thyroid cancer, skin cancer, lymphoma, melanoma or leukemia.

The antibody administered in step (b) is preferably specific for a tumour antigen associated with one or more of the above cancer types. Targets of interest for an antibody for use in the method include CD2, CD3, CD19, CD20, CD22, CD25, CD30, CD32, CD33, CD40, CD52, CD54, CD56, CD64, CD70, CD74, CD79, CD80, CD86, CD105, CD138, CD174, CD205, CD227, CD326, CD340, MUC16, GPNMB, PSMA, Cripto, ED-B, TMEFF2, EphA2, EphB2, FAP, av integrin, Mesothelin, EGFR, TAG-72, GD2, CA1X, 5T4, $\alpha 4\beta 7$ integrin, Her2. Other targets are cytokines, such as interleukins IL-I through IL- 13, tumour necrosis factors α & β, interferons α, β and γ, tumour growth factor Beta (TGF-β), colony stimulating factor (CSF) and granulocyte monocyte colony stimulating factor (GMCSF). See Human Cytokines: Handbook for Basic & Clinical Research (Aggrawal *et al.* eds., Blackwell Scientific, Boston, MA 1991). Other targets are hormones, enzymes, and intracellular and intercellular messengers, such as, adenyl cyclase, guanyl cyclase, and phospholipase C. Other targets of interest are leukocyte antigens, such as CD20, and CD33. Drugs may also be targets of interest. Target molecules can be human, mammalian or bacterial. Other targets are antigens, such as proteins, glycoproteins and carbohydrates from microbial pathogens, both viral and bacterial, and tumors. Still other targets are described in U.S. 4,366,241.

The antibody may be attached directly or indirectly to a cytotoxic moiety or to a detectable label. The antibody may be administered via one or more routes of administration using one or more of a variety of methods known in the art. The route and/or mode of administration will vary depending upon the desired results. Preferred routes of administration for antibodies include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection. Alternatively, an antibody can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration. Local administration is also preferred,

including peritumoral, juxtatumoral, intratumoral, intralesional, perilesional, intra cavity infusion, intravesicle administration, and inhalation.

A suitable dosage of an antibody of the invention may be determined by a skilled medical practitioner. Actual dosage levels of an antibody may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular antibody employed, the route of administration, the time of administration, the rate of excretion of the antibody, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A suitable dose of an antibody may be, for example, in the range of from about 0.1μg/kg to about 100mg/kg body weight of the patient to be treated. For example, a suitable dosage may be from about 1μg/kg to about 10mg/kg body weight per day or from about 10μg/kg to about 5 mg/kg body weight per day.

Dosage regimens may be adjusted to provide the optimum desired response (*e.g.,* a therapeutic response). For example, a single bolus may be administered, or step (b) of the method may comprise several divided doses administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation, provided the required interval between stesp (a) and (b) is not exceeded. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

The antibody of step (b) may be administered in combination with chemotherapy or radiation therapy. The method may further comprises the administration of an additional anti-cancer antibody or other therapeutic agent, which may be administered together with the antbody of step (b) in a single composition or in separate compositions as part of a combined therapy. For example, the antibody of step (b) may be administered before, after or concurrently with the other agent.

The antibody may be Abagovomab, Abciximab,Actoxumab, Adalimumab, Adecatumumab, Afelimomab, Afutuzumab, Alacizumab pegol, ALD518, Alemtuzumab,

Alirocumab, Altumomab pentetate, Amatuximab, Anatumomab mafenatox, Anrukinzumab, Apolizumab, Arcitumomab, Aselizumab, Atinumab, Atlizumab (= tocilizumab), Atorolimumab, Bapineuzumab, Basiliximab, Bavituximab, Bectumomab, Belimumab, Benralizumab, Bertilimumab, Besilesomab, Bevacizumab, Bezlotoxumab, Biciromab, Bimagrumab, Bivatuzumab mertansine, Blinatumomab, Blosozumab, Brentuximab vedotin, Briakinumab, Brodalumab, Canakinumab, Cantuzumab mertansine, Cantuzumab ravtansine, Caplacizumab, Capromab pendetide, Carlumab, Catumaxomab, CC49, Cedelizumab, Certolizumab pegol, Cetuximab, Ch.14.18, Citatuzumab bogatox, Cixutumumab, Clazakizumab, Clenoliximab, Clivatuzumab tetraxetan, Conatumumab, Concizumab, Crenezumab, CR6261, Dacetuzumab, Daclizumab, Dalotuzumab, Daratumumab, Demcizumab, Denosumab, Detumomab, Dorlimomab aritox, Drozitumab, Duligotumab, Dupilumab, Dusigitumab, Ecromeximab, Eculizumab, Edobacomab, Edrecolomab, Efalizumab, Efungumab, Elotuzumab Elsilimomab, Enavatuzumab, Enlimomab pegol, Enokizumab, Enoticumab, Ensituximab, Epitumomab cituxetan, Epratuzumab, Erlizumab, Ertumaxomab, Etaracizumab, Etrolizumab, Evolocumab, Exbivirumab, Fanolesomab, Faralimomab Farletuzumab, Fasinumab, FBTA05, Felvizumab, Fezakinumab, Ficlatuzumab, Figitumumab, Flanvotumab, Fontolizumab, Foralumab, Foravirumab, Fresolimumab, Fulranumab, Futuximab, Galiximab,Ganitumab, Gantenerumab, Gavilimomab, Gemtuzumab ozogamicin, Gevokizumab, Girentuximab,Glembatumumab vedotin, Golimumab, Gomiliximab,GS6624, Ibalizumab, Ibritumomab tiuxetan, Icrucumab, Igovomab, Imciromab, Imgatuzumab, Inclacumab, Indatuximab ravtansine, Infliximab, Intetumumab, Inolimomab, Inotuzumab ozogamicin, Ipilimumab, Iratumumab, Itolizumab, Ixekizumab, Keliximab, Labetuzumab, Lampalizumab, Lebrikizumab, Lemalesomab, Lerdelimumab, Lexatumumab, Libivirumab, Ligelizumab, Lintuzumab, Lirilumab, Lodelcizumab, Lorvotuzumab mertansine, Lucatumumab, Lumiliximab, Mapatumumab, Maslimomab, Mavrilimumab, Matuzumab, Mepolizumab, Metelimumab, Milatuzumab, Minretumomab, Mitumomab, Mogamulizumab, Morolimumab, Motavizumab, Moxetumomab pasudotox, Muromonab-CD3, Nacolomab tafenatox, Namilumab, Naptumomab estafenatox, Narnatumab, Natalizumab, Nebacumab, Necitumumab, Nerelimomab, Nesvacumab, Nimotuzumab, Nivolumab, Nofetumomab merpentan, Obinutuzumab, Ocaratuzumab, Ocrelizumab, Odulimomab, Ofatumumab, Olaratumab, Olokizumab, Omalizumab, Onartuzumab, Oportuzumab monatox, Oregovomab, Orticumab, Otelixizumab, Oxelumab, Ozanezumab, Ozoralizumab, Pagibaximab, Palivizumab, Panitumumab, Panobacumab, Parsatuzumab, Pascolizumab, Pateclizumab, Patritumab, Pemtumomab, Perakizumab, Pertuzumab,

Pexelizumab, Pidilizumab, Pinatuzumab vedotin, Pintumomab, Placulumab, Polatuzumab vedotin, Ponezumab, Priliximab, Pritoxaximab, Pritumumab, PRO 140, Quilizumab, Racotumomab, Radretumab, Rafivirumab, Ramucirumab, Ranibizumab,Raxibacumab, Regavirumab, Reslizumab, Rilotumumab, Rituximab, Robatumumab, Roledumab, Romosozumab, Rontalizumab, Rovelizumab, Ruplizumab, Samalizumab, Sarilumab, Satumomab pendetide, Secukinumab, Seribantumab, Setoxaximab, Sevirumab, Sibrotuzumab, Sifalimumab, Siltuximab, Simtuzumab, Siplizumab, Sirukumab, Solanezumab, Solitomab, Sonepcizumab, Sontuzumab, Stamulumab, Sulesomab, Suvizumab, Tabalumab, Tacatuzumab tetraxetan, Tadocizumab, Talizumab, Tanezumab, Taplitumomab paptox, Tefibazumab, Telimomab aritox, Tenatumomab, Teneliximab, Teplizumab, Teprotumumab, TGN1412, Ticilimumab (= tremelimumab), Tildrakizumab, Tigatuzumab, TNX-650, Tocilizumab (= atlizumab), Toralizumab, Tositumomab, Tralokinumab, Trastuzumab, TRBS07, Tregalizumab, Tremelimumab Tucotuzumab celmoleukin, Tuvirumab, Ublituximab, Urelumab, Urtoxazumab, Ustekinumab, Vapaliximab, Vatelizumab, Vedolizumab, Veltuzumab,Vepalimomab Vesencumab, Visilizumab, Volociximab, Vorsetuzumab mafodotin, Votumumab, Zalutumumab, Zanolimumab, Zatuximab, Ziralimumab or Zolimomab aritox.

Preferred antibodies include Natalizumab, Vedolizumab, Belimumab, Atacicept, Alefacept, Otelixizumab, Teplizumab, Rituximab, Ofatumumab, Ocrelizumab, Epratuzumab, Alemtuzumab, Abatacept, Eculizamab, Omalizumab, Canakinumab, Meplizumab, Reslizumab, Tocilizumab, Ustekinumab, Briakinumab, Etanercept, Inlfliximab, Adalimumab, Certolizumab pegol, Golimumab, Trastuzumab, Gemtuzumab, Ozogamicin, Ibritumomab, Tiuxetan, Tostitumomab, Cetuximab, Bevacizumab, Panitumumab, Denosumab, Ipilimumab, Brentuximab and Vedotin.

The therapy of which the benefit is improved is typically an organ transplant. The organ may be selected from kidney, liver, heart, pancreas, lung, or small intestine. The subject to be treated may preferably be sensitized or highly sensitised. By "sensitized" it is meant that the subject has developed antibodies to human major histocompatibility (MHC) antigens (also referred to as human leukocyte antigens (HLA)). The anti-HLA antibodies originate from allogenically sensitized B-cells and are usually present in patients that have previously been sensitized by blood transfusion, previous transplantation or pregnancy (Jordan *et al.,* 2003).

Whether or not a potential transplant recipient is sensitized may be determined by any suitable method. For example, a Panel Reactive Antibody (PRA) test may be used to

determine if a recipient is sensitized. A PRA score >30% is typically taken to mean that the patient is "high immulogic risk" or "sensitized". Alternatively, a cross match test may be conducted, in which a sample of the potential transplant donor's blood is mixed with that of the intended recipient. A positive cross-match means that the recipient has antibodies which react to the donor sample, indicating that the recipient is sensitized and transplantation should not occur. Cross-match tests are typically conducted as a final check immediately prior to transplantation.

The presence of high titer antibodies against MHC antigens of the potential donor (i.e. donor specific antibodies (DSA)) is a direct contraindication to transplantation because of the risk of acute antibody-mediated rejection. In short, sensitization to donor MHC antigens hampers the identification of a suitable donor. A positive cross-match test is an unambiguous barrier to transplantation. Since approximately one third of patients waiting for kidney transplantation are sensitized, with as many as 15% being highly sensitized, this leads to an accumulation of patients waiting for transplant. In the US, the median time on the waiting list for renal transplantation in 2001–2002 was 1329 days for those with Panel Reactive Antibody (PRA) score 0–9%, 1920 days for those with PRA 10–79%, and 3649 days for those with PRA 80% or greater (OPTN-database, 2011).

One accepted strategy to overcome the DSA barrier is to apply plasma exchange or immune adsorption, often in combination with e.g. intravenous gamma globulin (IVIg) or Rituximab, to lower the levels of DSA to a level where transplantation can be considered (Jordan et al., 2004; Montgomery et al., 2000; Vo et al., 2008a; Vo et al., 2008b). However, plasma exchange, immune adsorption and IVIg treatments have the disadvantage of being inefficient and requiring rigorous planning since they involve repeated treatments over an extended period of time. When an organ from a deceased donor becomes available it has to be transplanted within hours since prolonged cold ischemia time is one of the most important risk factors for delayed graft function and allograft loss in renal transplantation (Ojo et al., 1997).

By contrast, the method of the present invention allows the rapid, temporary and safe removal of DSAs in a potential transplant recipient. Administering the polypeptide of the invention just prior to transplantation has the capacity to effectively desensitize a highly sensitized patient, thereby allowing transplantation and avoiding acute antibody-mediated rejection. A single dose of polypeptide prior to transplantation will enable transplantation of thousands of patients with donor specific IgG antibodies.

In the context of this embodiment, the method may be alternatively described as a method for the treatment of organ failure in a subject, the method comprising (a) administering to the subject a polypeptide of the invention and (b) subsequently transplanting a replacement organ into the subject; wherein:

- the amount of said polypeptide administered is sufficient to cleave substantially all IgG molecules present in the plasma of the subject; and
- steps (a) and (b) are separated by a time interval of at least 2 hours and at most 21 days.

In other words, this embodiment may be described as a method for preventing rejection of a transplanted organ in a subject, particularly acute antibody-mediated transplant rejection, the method comprising, at least 2 hours and at most 21 days prior to transplantation of the organ, administering to the subject a polypeptide of the invention, wherein the amount of said polypeptide administered is sufficient to cleave substantially all IgG molecules present in the plasma of the subject. The invention also provides use of the polypeptide of the invention in such a method of treating organ failure or preventing transplant rejection, particularly acute antibody-mediated transplant rejection. The invention also provides use of the polypeptide of the invention in the manufacture of a medicament for the treatment of organ failure or for the prevention of transplant rejection by such a method. In this embodiment, the method of the invention may additionally comprise a step conducted at or immediately prior to transplantation, which step comprises induction suppression of T cells and/or B cells in the patient. Said induction suppression may typically comprise administering an effective amount of an agent which kills or inhibits T cells, and/or administering an effective amount of an agent which kills or inhibits B cells. Agents which kill or inhibit T cells include Muromonab, Basiliximab, Daclizumab, an antithymocyte globulin (ATG) antibody and a lymphocyte immune globulin, anti-thymocyte globulin preparation (ATGAM). Rituximab is known to kill or inhibit B cells.

Examples

Unless indicated otherwise, the methods used are standard biochemistry and molecular biology techniques. Examples of suitable methodology textbooks include Sambrook et al., Molecular Cloning, A Laboratory Manual (1989) and Ausubel et al., Current Protocols in Molecular Biology (1995), John Wiley and Sons, Inc.

Example 1 - Design of polypeptides, production and purification

The mature IdeS molecule was analysed and regions suitable for mutation were identified. In some cases an *in silico* assessment was used to evaluate the likely outcome of a mutation. Having decided on the sequence of each polypeptide, cDNA encoding each polypeptide were generated at GeneCust, Luxembourg either by site-directed mutation of a starting sequence or synthesis depending on the number of mutations introduced. cDNA were sequenced and transferred to the pET9a expression vector (Novagene) in frame with a C-terminal 6x His-tag, joined to the C-terminus by a short glycine linker (3x Gly). N terminal methionine was added to improve bacterial expression. The plasmids were transformed (heat-shock) into *E. coli* BL21(DE3) (Stratagene) and seeded on LB agarose plates containing 30 µg/ml kanamycin. Single colonies were picked and overnight cultures (3 ml LB-medium) were started at 37°C, 250 rpm. The following day glycerol stocks were prepared and 10 ml TB-medium supplemented with 30 µg/ml kanamycin and anti-foam were inoculated with overnight culture and grown until OD 0.6-0.8 (37°C, 300 rpm). At this point IPTG (1 mM) was added and cultures were continued for 1 hour prior to harvest of the bacteria by centrifugation. The pellets were washed in PBS and frozen at -20°C. A freeze-thaw protocol for bacterial lysis was used (three freeze/thaw cycles in 1 ml PBS each) and the proteins were purified using Ni-NTA pre-packed spin-columns (Pierce). After purification the eluted proteins were activated with 10 mM DTT prior to buffer exchange (3 volumes PBS in MWCO 9K Millipore cfg devises). The purity and stability of each protein was evaluated using sodium dodecyl sulphate polyacrylamide gel electrophoreses (SDS-PAGE) stainless 12% Mini-PROTEAN®TGX™ precast gel (Biorad) SDS-PAGE.

The following table summarises the changes made for each tested polypeptide relative to mature IdeZ or IdeS/Z, not including the N terminal methionine and his tag. Thus, the sequence of each polypeptide used in the experiments described herein typically comprises the sequence of the SEQ ID NO as indicated in the table, plus an additional N terminal methionine and a his tag joined to the C terminal end by a short glycine linker.

Internal reference	Modifications relative to IdeZ (SEQ ID NO: 4) (positions correspond to SEQ ID NO: 3)	SEQ ID NO
pCART197	H84N, N138R, A147E,D150R, N162E, N171Y, N205K, D226N, Q251E, E255K, A312K, S349N	6
pCART198	A93T, D95N, Q140E, R165K, D166E, A174T, D226N, L237F, N239E, N243K, N282D, E288K, H315K, K347Q	7
pCART200	D36_I53replacedS31_V48 of SEQ 2 i.e. SFSANQEI RYSEVTPYHV replaces DYQRNATE AYAKEVPHQI	8
pCART201	D35_T54del	9

pCART202	R70T, Y71del, N72Q, N73G	10
pCART203	L64_T65del, R70T, Y71del, N72Q, N73G	11
pCART204	R70T, Y71del	12
pCART206	L64_T65del, R70T, Y71del, N72Q, N73G, F137I	13
pCART207	L64_T65del, R70T, Y71del, N72Q, N73G, N138R	14
pCART208	L64_T65del, R70T, Y71del, N72Q, N73G, F137I, N138R	15
pCART210	L64_T65del, R70T, Y71del, N72Q, N73G, H84N, N138R, N162E, N205K, D226N	16
pCART217	D35_T54del, L64_T65del, R70T, Y71del, N72Q, N73G, N138R, D226N	17
pCART219	L64_T65del, R70T, Y71del, N72Q, N73G, K97A, N138R, D226N	18
pCART226	D35_T54del, L64_T65del, R70T, Y71del, N72Q, N73G, K97A, N138R, D226N	19
pCART229	L64_T65del, R70T, Y71del, N72Q, N73G, N138R, D226N	20

Internal reference	Modifications relative to IdeS/Z (SEQ ID NO: 5) (positions correspond to SEQ ID NO: 3)	SEQ ID NO
pCART191	R70T, Y71del, N72Q, N73G, D140E, G171Y, R175K, R176H, I177L, S267R	21
pCART192	R70T, Y71del, N72Q, N73G, N138R, D140E, K147E, D150R, N162E, G171Y, R175K, R176H, I177L, E206K, Q249S, K253N, S267R, A310K, S347N	22
pCART193	R70T, Y71del, N72Q, N73G, A93T, D95N, N99D, D140E, N141Q, K147E, N162E, G171Y, A174T, R175K, R176H, I177L, N237E, N241K, D242E, T245D, I246L, K253E, S267R, E286K, H311A, H313K, Q344N, K345Q, L346T	23
pCART194	R70T, Y71del, N72Q, N73G, A93T, D95N, N99D, N138R, D140E, N141Q, K147E, D150R, N162E, G171Y, A174T, R175K, R176H, I177L, E206K, N237E, N241K, D242E, T245D, I246L, Q249E, S267R, N280D, E286K, A310K, H311A, H313K, Q344N, K345Q, L346T, S347N	24
pCART205	L64_65del, R70T, Y71del, N72Q, N73G, F77I, N138R, D140E, N141Q	25

As controls, versions of IdeS, IdeZ and IdeS/Z were produced using the same methodology as described above. These versions are referred to herein as pCART124, pCART144 and pCART145 respectively.

pCART124 comprises the sequence of SEQ ID NO: 2 plus an additional N terminal methionine and a his tag joined to the C terminal end by a short glycine linker.

The sequence of pCART124 is provided below:

MDSFSANQEIRYSEVTPYHVTSVWTKGVTPPANFTQGEDVFHAPYVANQGWYDITKTFNGKDDLLCGAATAGNML
HWWFDQNKDQIKRYLEEHPEKQKINFNGEQMFDVKEAIDTKNHQLDSKLFEYFKEKAFPYLSTKHLGVFPDHVID
MFINGYRLSLTNHGPTPVKEGSKDPRGGIFDAVFTRGDQSKLLTSRHDFKEKNLKEISDLIKKELTEGKALGLSH
TYANVRINHVINLWGADFDSNGNLKAIYVTDSDSNASIGMKKYFVGVNSAGKVAISAKEIKEDNIGAQVLGLFTL
STGQDSWNQTNGGGHHHHHH (SEQ ID NO: 26)

pCART144 comprises the sequence of SEQ ID NO: 4 plus an additional N terminal methionine and a his tag joined to the C terminal end by a short glycine linker.

The sequence of pCART144 is provided below:

MDDYQRNATEAYAKEVPHQITSVWTKGVTPLTPEQFRYNNEDVIHAPYLAHQGWYDITKAFDGKDNLLCGAATAG
NMLHWWFDQNKTEIEAYLSKHPEKQKIIFNNQELFDLKAAIDTKDSQTNSQLFNYFRDKAFPNLSARQLGVMPDL

VLDMFINGYYLNVFKTQSTDVNRPYQDKDKRGGIFDAVFTRGDQTTLLTARHDLKNKGLNDISTIIKQELTEGRA
LALSHTYANVSISHVINLWGADFNAEGNLEAIYVTDSDANASIGMKKYFVGINAHGHVAISAKKIEGENIGAQVL
GLFTLSSGKDIWQKLSGGGHHHHHH (SEQ ID NO: 27)

pCART145 comprises the sequence of SEQ ID NO: 5 plus an additional N terminal methionine and a his tag joined to the C terminal end by a short glycine linker.

The sequence of pCART145 is provided below:

MDDYQRNATEAYAKEVPHQITSVWTKGVTPLTPEQFRYNNEDVFHAPYVANQGWYDITKAFDGKDNLLCGAATAG
NMLHWWFDQNKDQIKRYLEEHPEKQKINFNGDNMFDVKKAIDTKNHQLDSKLFNYFKEKAFPGLSARRIGVFPDH
VIDMFINGYRLSLTNHGPTPVKEGSKDPRGGIFDAVFTRGNQSKLLTSRHDFKNKNLNDISTIIKQELTKGKALG
LSHTYANVSINHVINLWGADFNAEGNLEAIYVTDSDSNASIGMKKYFVGVNAHGHVAISAKKIEGENIGAQVLGL
FTLSTGQDSWQKLSGGGHHHHHH (SEQ ID NO: 28)

IdeS lacking tag was also independently produced to GMP standard using automated multistep chromatographic purification, for use as a further control. This polypeptide is referred to herein as BX1001865.

The cDNA sequence used to produce each of the tested polypeptides and pCART124, pCART144 and pCART145 is provided below. Each cDNA sequence includes at the 3' end a codon for the N terminal methionine (ATG) and, prior to the stop codon (TAA) at the 5' end, codons for the glycine linker and the histidine tag.

pCART124 (IdeS; SEQ ID NO: 33)

ATGGATAGTTTTTCTGCTAATCAAGAGATTAGATATTCGGAAGTAACACCTTATCACGTTACTTCCGTTTGGACC
AAAGGAGTTACTCCTCCAGCAAACTTCACTCAAGGTGAAGATGTTTTTCACGCTCCTTATGTTGCTAACCAAGGA
TGGTATGATATTACCAAAACATTCAATGGAAAAGACGATCTTCTTTGCGGGGCTGCCACAGCAGGGAATATGCTT
CACTGGTGGTTCGATCAAAACAAAGACCAAATTAAACGTTATTTGGAAGAGCATCCAGAAAAGCAAAAAATAAAC
TTCAATGGCGAACAGATGTTTGACGTAAAAGAAGCTATCGACACTAAAAACCACCAGCTAGATAGTAAATTATTT
GAATATTTTAAAGAAAAAGCTTTCCCTTATCTATCTACTAAACACCTAGGAGTTTTCCCTGATCATGTAATTGAT
ATGTTCATTAACGGCTACCGCCTTAGTCTAACTAACCACGGTCCAACGCCAGTAAAAGAAGGTAGTAAAGATCCC
CGAGGTGGTATTTTTGACGCCGTATTTACAAGAGGTGATCAAAGTAAGCTATTGACAAGTCGTCATGATTTTAAA
GAAAAAAATCTCAAAGAAATCAGTGATCTCATTAAGAAAGAGTTAACCGAAGGCAAGGCTCTAGGCCTATCACAC
ACCTACGCTAACGTACGCATCAACCATGTTATAAACCTGTGGGGAGCTGACTTTGATTCTAACGGGAACCTTAAA
GCTATTTATGTAACAGACTCTGATAGTAATGCATCTATTGGTATGAAGAAATACTTTGTTGGTGTTAATTCCGCT
GGAAAAGTAGCTATTTCTGCTAAAGAAATAAAAGAAGATAATATAGGTGCTCAAGTACTAGGGTTATTTACACTT
TCAACAGGGCAAGATAGTTGGAATCAGACCAATGGCGGTGGCCATCATCACCATCACCACTAA

pCART144 (IdeZ; SEQ ID NO: 34)

ATGGACGATTACCAAAGGAATGCTACGGAAGCTTATGCCAAAGAAGTACCACATCAGATCACTTCTGTATGGACC
AAAGGTGTTACACCACTAACACCCGAGCAGTTTCGATATAATAACGAAGATGTGATCCATGCGCCATATCTTGCT
CATCAAGGCTGGTACGATATCACCAAGGCCTTCGATGGGAAGGATAATCTCTTGTGTGGCGCAGCAACGGCAGGT
AATATGCTGCATTGGTGGTTTGATCAAAATAAAACAGAGATTGAAGCCTATTTAAGTAAACACCCTGAAAAGCAA
AAAATCATTTTTAACAACCAAGAGCTATTTGATTTGAAAGCTGCTATCGATACCAAGGACAGTCAAACCAATAGT
CAGCTTTTTAATTATTTTAGAGATAAAGCCTTTCCAAATCTATCAGCACGTCAACTCGGGGTTATGCCTGATCTT
GTTCTAGATATGTTTATCAATGGTTACTACTTAAATGTGTTTAAAACACAGTCTACTGATGTCAATCGACCTTAT
CAGGACAAGGACAAACGAGGTGGTATTTTCGATGCTGTTTTCACCAGAGGAGATCAGACAACGCTCTTGACAGCT
CGTCATGATTTAAAAAATAAAGGACTAAATGACATCAGCACCATTATCAAGCAAGAACTGACTGAAGGAAGAGCC
CTTGCTTTATCACATACCTACGCCAATGTTAGCATTAGCCATGTGATTAACTTGTGGGGAGCTGATTTTAATGCT
GAAGGAAACCTTGAGGCCATCTATGTCACAGACTCAGATGCTAATGCGTCTATTGGTATGAAAAAATATTTTGTC
GGCATTAATGCTCATGGACATGTCGCCATTTCTGCCAAGAAAATAGAAGGAGAAAACATTGGCGCTCAAGTATTA

GGCTTATTTACGCTTTCCAGTGGCAAGGACATTTGGCAGAAACTGAGCGGCGGTGGCCATCATCACCATCACCAC
TAA

pCART145 (IdeS/Z; SEQ ID NO: 35)

ATGGATGATTATCAGCGCAACGCGACCGAAGCGTATGCGAAAGAAGTGCCGCATCAGATTACCAGCGTGTGGACC
AAAGGCGTGACCCCGCTGACCCCGGAACAGTTTCGCTATAACAACGAAGATGTGTTTCATGCGCCGTATGTGGCG
AACCAGGGCTGGTATGATATTACCAAAGCGTTTGATGGCAAAGATAACCTGCTGTGCGGCGCGGCGACCGCGGGC
AACATGCTGCATTGGTGGTTTGATCAGAACAAAGATCAGATTAAACGCTATCTGGAAGAACATCCGGAAAAACAG
AAAATTAACTTTAACGGCGATAACATGTTTGATGTGAAAAAAGCGATTGATACCAAAAACCATCAGCTGGATAGC
AAACTGTTTAACTATTTTAAAGAAAAAGCGTTTCCGGGCCTGAGCGCGCGCCGCATTGGCGTGTTTCCGGATCAT
GTGATTGATATGTTTATTAACGGCTATCGCCTGAGCCTGACCAACCATGGCCCGACCCCGGTGAAAGAAGGCAGC
AAAGATCCGCGCGGCGGCATTTTTGATGCGGTGTTTACCCGCGGCAACCAGAGCAAACTGCTGACCAGCCGCCAT
GATTTTAAAAACAAAAACCTGAACGATATTAGCACCATTATTAAACAGGAACTGACCAAAGGCAAAGCGCTGGGC
CTGAGCCATACCTATGCGAACGTGAGCATTAACCATGTGATTAACCTGTGGGGCGCGGATTTTAACGCGGAAGGC
AACCTGGAAGCGATTTATGTGACCGATAGCGATAGCAACGCGAGCATTGGCATGAAAAAATATTTTGTGGGCGTG
AACGCGCATGGCCATGTGGCGATTAGCGCGAAAAAAATTGAAGGCGAAAACATTGGCGCGCAGGTGCTGGGCCTG
TTTACCCTGAGCACCGGCCAGGATAGCTGGCAGAAACTGAGCGGCGGTGGCCATCATCACCATCACCACTAA

pCART197 (SEQ ID NO: 36)

ATGGATGATTATCAGCGCAACGCGACCGAAGCGTATGCGAAAGAAGTGCCGCATCAGATTACCAGCGTGTGGACC
AAAGGCGTGACCCCGCTGACCCCGGAACAGTTTCGCTATAACAACGAAGATGTGATTCATGCGCCGTATCTGGCG
AACCAGGGCTGGTATGATATTACCAAAGCGTTTGATGGCAAAGATAACCTGCTGTGCGGCGCGGCGACCGCGGGC
AACATGCTGCATTGGTGGTTTGATCAGAACAAAACCGAAATTGAAGCGTATCTGAGCAAACATCCGGAAAAACAG
AAAATTATTTTTCGCAACCAGGAACTGTTTGATCTGAAAGAAGCGATTCGCACCAAAGATAGCCAGACCAACAGC
CAGCTGTTTGAATATTTTCGCGATAAAGCGTTTCCGTATCTGAGCGCGCGCCAGCTGGGCGTGATGCCGGATCTG
GTGCTGGATATGTTTATTAACGGCTATTATCTGAACGTGTTTAAAACCCAGAGCACCGATGTGAAACGCCCGTAT
CAGGATAAAGATAAACGCGGCGGCATTTTTGATGCGGTGTTTACCCGCGGCAACCAGACCACCCTGCTGACCGCG
CGCCATGATCTGAAAAACAAAGGCCTGAACGATATTAGCACCATTATTAAAGAAGAACTGACCAAAGGCCGCGCG
CTGGCGCTGAGCCATACCTATGCGAACGTGAGCATTAGCCATGTGATTAACCTGTGGGGCGCGGATTTTAACGCG
GAAGGCAACCTGGAAGCGATTTATGTGACCGATAGCGATGCGAACGCGAGCATTGGCATGAAAAAATATTTTGTG
GGCATTAACAAACATGGCCATGTGGCGATTAGCGCGAAAAAAATTGAAGGCGAAAACATTGGCGCGCAGGTGCTG
GGCCTGTTTACCCTGAGCAGCGGCAAAGATATTTGGCAGAAACTGAACGGCGGTGGCCATCATCACCATCACCAC
TAA

pCART198 (SEQ ID NO: 37)

ATGGATGATTATCAGCGCAACGCGACCGAAGCGTATGCGAAAGAAGTGCCGCATCAGATTACCAGCGTGTGGACC
AAAGGCGTGACCCCGCTGACCCCGGAACAGTTTCGCTATAACAACGAAGATGTGATTCATGCGCCGTATCTGGCG
CATCAGGGCTGGTATGATATTACCAAAACCTTTAACGGCAAAGATAACCTGCTGTGCGGCGCGGCGACCGCGGGC
AACATGCTGCATTGGTGGTTTGATCAGAACAAAACCGAAATTGAAGCGTATCTGAGCAAACATCCGGAAAAACAG
AAAATTATTTTTAACAACGAAGAACTGTTTGATCTGAAAGCGGCGATTGATACCAAAGATAGCCAGACCAACAGC
CAGCTGTTTAACTATTTTAAAGAAAAAGCGTTTCCGAACCTGAGCACCCGCCAGCTGGGCGTGATGCCGGATCTG
GTGCTGGATATGTTTATTAACGGCTATTATCTGAACGTGTTTAAAACCCAGAGCACCGATGTGAACCGCCCGTAT
CAGGATAAAGATAAACGCGGCGGCATTTTTGATGCGGTGTTTACCCGCGGCAACCAGACCACCCTGCTGACCGCG
CGCCATGATTTTAAAGAAAAAGGCCTGAAAGATATTAGCACCATTATTAAACAGGAACTGACCGAAGGCCGCGCG
CTGGCGCTGAGCCATACCTATGCGAACGTGAGCATTAGCCATGTGATTAACCTGTGGGGCGCGGATTTTGATGCG
GAAGGCAACCTGAAAGCGATTTATGTGACCGATAGCGATGCGAACGCGAGCATTGGCATGAAAAAATATTTTGTG
GGCATTAACGCGCATGGCAAAGTGGCGATTAGCGCGAAAAAAATTGAAGGCGAAAACATTGGCGCGCAGGTGCTG
GGCCTGTTTACCCTGAGCAGCGGCAAAGATATTTGGCAGCAGCTGAGCGGCGGTGGCCATCATCACCATCACCAC
TAA

pCART200 (SEQ ID NO: 38)

ATGGATAGCTTTAGCGCGAACCAGGAAATTCGCTATAGCGAAGTGACCCCGTATCATGTGACCAGCGTGTGGACC
AAAGGCGTGACCCCGCTGACCCCGGAACAGTTTCGCTATAACAACGAAGATGTGATTCATGCGCCGTATCTGGCG
CATCAGGGCTGGTATGATATTACCAAAGCGTTTGATGGCAAAGATAACCTGCTGTGCGGCGCGGCGACCGCGGGC
AACATGCTGCATTGGTGGTTTGATCAGAACAAAACCGAAATTGAAGCGTATCTGAGCAAACATCCGGAAAAACAG
AAAATTATTTTTAACAACCAGGAACTGTTTGATCTGAAAGCGGCGATTGATACCAAAGATAGCCAGACCAACAGC
CAGCTGTTTAACTATTTTCGCGATAAAGCGTTTCCGAACCTGAGCGCGCGCCAGCTGGGCGTGATGCCGGATCTG

GTGCTGGATATGTTTATTAACGGCTATTATCTGAACGTGTTTAAAACCCAGAGCACCGATGTGAACCGCCCGTAT
CAGGATAAAGATAAACGCGGCGGCATTTTTGATGCGGTGTTTACCCGCGGCGATCAGACCACCCTGCTGACCGCG
CGCCATGATCTGAAAAACAAAGGCCTGAACGATATTAGCACCATTATTAAACAGGAACTGACCGAAGGCCGCGCG
CTGGCGCTGAGCCATACCTATGCGAACGTGAGCATTAGCCATGTGATTAACCTGTGGGGCGCGGATTTTAACGCG
GAAGGCAACCTGGAAGCGATTTATGTGACCGATAGCGATGCGAACGCGAGCATTGGCATGAAAAAATATTTTGTG
GGCATTAACGCGCATGGCCATGTGGCGATTAGCGCGAAAAAAATTGAAGGCGAAAACATTGGCGCGCAGGTGCTG
GGCCTGTTTACCCTGAGCAGCGGCAAAGATATTTGGCAGAAACTGAGCGGCGGTGGCCATCATCACCATCACCAC
TAA

pCART201 (SEQ ID NO: 39)
ATGAGCGTGTGGACCAAAGGCGTGACCCCGCTGACCCCGGAACAGTTTCGCTATAACAACGAAGATGTGATTCAT
GCGCCGTATCTGGCGCATCAGGGCTGGTATGATATTACCAAAGCGTTTGATGGCAAAGATAACCTGCTGTGCGGC
GCGGCGACCGCGGGCAACATGCTGCATTGGTGGTTTGATCAGAACAAAACCGAAATTGAAGCGTATCTGAGCAAA
CATCCGGAAAAACAGAAAATTATTTTTAACAACCAGGAACTGTTTGATCTGAAAGCGGCGATTGATACCAAAGAT
AGCCAGACCAACAGCCAGCTGTTTAACTATTTTCGCGATAAAGCGTTTCCGAACCTGAGCGCGCGCCAGCTGGGC
GTGATGCCGGATCTGGTGCTGGATATGTTTATTAACGGCTATTATCTGAACGTGTTTAAAACCCAGAGCACCGAT
GTGAACCGCCCGTATCAGGATAAAGATAAACGCGGCGGCATTTTTGATGCGGTGTTTACCCGCGGCGATCAGACC
ACCCTGCTGACCGCGCGCCATGATCTGAAAAACAAAGGCCTGAACGATATTAGCACCATTATTAAACAGGAACTG
ACCGAAGGCCGCGCGCTGGCGCTGAGCCATACCTATGCGAACGTGAGCATTAGCCATGTGATTAACCTGTGGGGC
GCGGATTTTAACGCGGAAGGCAACCTGGAAGCGATTTATGTGACCGATAGCGATGCGAACGCGAGCATTGGCATG
AAAAAATATTTTGTGGGCATTAACGCGCATGGCCATGTGGCGATTAGCGCGAAAAAAATTGAAGGCGAAAACATT
GGCGCGCAGGTGCTGGGCCTGTTTACCCTGAGCAGCGGCAAAGATATTTGGCAGAAACTGAGCGGCGGTGGCCAT
CATCACCATCACCACTAA

pCART202 (SEQ ID NO: 40)
ATGGACGATTACCAAAGGAATGCTACGGAAGCTTATGCCAAAGAAGTACCACATCAGATCACTTCTGTATGGACC
AAAGGTGTTACACCACTAACACCCGAGCAGTTTACTCAAGGTGAAGATGTGATCCATGCGCCATATCTTGCTCAT
CAAGGCTGGTACGATATCACCAAGGCCTTCGATGGGAAGGATAATCTCTTGTGTGGCGCAGCAACGGCAGGTAAT
ATGCTGCATTGGTGGTTTGATCAAAATAAAACAGAGATTGAAGCCTATTTAAGTAAACACCCTGAAAAGCAAAAA
ATCATTTTTAACAACCAAGAGCTATTTGATTTGAAAGCTGCTATCGATACCAAGGACAGTCAAACCAATAGTCAG
CTTTTTAATTATTTTAGAGATAAAGCCTTTCCAAATCTATCAGCACGTCAACTCGGGGTTATGCCTGATCTTGTT
CTAGATATGTTTATCAATGGTTACTACTTAAATGTGTTTAAAACACAGTCTACTGATGTCAATCGACCTTATCAG
GACAAGGACAAACGAGGTGGTATTTTCGATGCTGTTTTCACCAGAGGAGATCAGACAACGCTCTTGACAGCTCGT
CATGATTTAAAAAATAAAGGACTAAATGACATCAGCACCATTATCAAGCAAGAACTGACTGAAGGAAGAGCCCTT
GCTTTATCACATACCTACGCCAATGTTAGCATTAGCCATGTGATTAACTTGTGGGGAGCTGATTTTAATGCTGAA
GGAAACCTTGAGGCCATCTATGTCACAGACTCAGATGCTAATGCGTCTATTGGTATGAAAAAATATTTTGTCGGC
ATTAATGCTCATGGACATGTCGCCATTTCTGCCAAGAAAATAGAAGGAGAAAACATTGGCGCTCAAGTATTAGGC
TTATTTACGCTTTCCAGTGGCAAGGACATTTGGCAGAAACTGAGCGGCGGTGGCCATCATCACCATCACCACTAA

pCART203 (SEQ ID NO: 41)
ATGGACGATTACCAAAGGAATGCTACGGAAGCTTATGCCAAAGAAGTACCACATCAGATCACTTCTGTATGGACC
AAAGGTGTTACACCACCCGAGCAGTTTACTCAAGGTGAAGATGTGATCCATGCGCCATATCTTGCTCATCAAGGC
TGGTACGATATCACCAAGGCCTTCGATGGGAAGGATAATCTCTTGTGTGGCGCAGCAACGGCAGGTAATATGCTG
CATTGGTGGTTTGATCAAAATAAAACAGAGATTGAAGCCTATTTAAGTAAACACCCTGAAAAGCAAAAAATCATT
TTTAACAACCAAGAGCTATTTGATTTGAAAGCTGCTATCGATACCAAGGACAGTCAAACCAATAGTCAGCTTTTT
AATTATTTTAGAGATAAAGCCTTTCCAAATCTATCAGCACGTCAACTCGGGGTTATGCCTGATCTTGTTCTAGAT
ATGTTTATCAATGGTTACTACTTAAATGTGTTTAAAACACAGTCTACTGATGTCAATCGACCTTATCAGGACAAG
GACAAACGAGGTGGTATTTTCGATGCTGTTTTCACCAGAGGAGATCAGACAACGCTCTTGACAGCTCGTCATGAT
TTAAAAAATAAAGGACTAAATGACATCAGCACCATTATCAAGCAAGAACTGACTGAAGGAAGAGCCCTTGCTTTA
TCACATACCTACGCCAATGTTAGCATTAGCCATGTGATTAACTTGTGGGGAGCTGATTTTAATGCTGAAGGAAAC
CTTGAGGCCATCTATGTCACAGACTCAGATGCTAATGCGTCTATTGGTATGAAAAAATATTTTGTCGGCATTAAT
GCTCATGGACATGTCGCCATTTCTGCCAAGAAAATAGAAGGAGAAAACATTGGCGCTCAAGTATTAGGCTTATTT
ACGCTTTCCAGTGGCAAGGACATTTGGCAGAAACTGAGCGGCGGTGGCCATCATCACCATCACCACTAA

pCART204 (SEQ ID NO: 42)
ATGGACGATTACCAAAGGAATGCTACGGAAGCTTATGCCAAAGAAGTACCACATCAGATCACTTCTGTATGGACC
AAAGGTGTTACACCACCCGAGCAGTTTCGATATAATAACGAAGATGTGATCCATGCGCCATATCTTGCTCATCAA
GGCTGGTACGATATCACCAAGGCCTTCGATGGGAAGGATAATCTCTTGTGTGGCGCAGCAACGGCAGGTAATATG
CTGCATTGGTGGTTTGATCAAAATAAAACAGAGATTGAAGCCTATTTAAGTAAACACCCTGAAAAGCAAAAAATC
ATTTTTAACAACCAAGAGCTATTTGATTTGAAAGCTGCTATCGATACCAAGGACAGTCAAACCAATAGTCAGCTT
TTTAATTATTTTAGAGATAAAGCCTTTCCAAATCTATCAGCACGTCAACTCGGGGTTATGCCTGATCTTGTTCTA

GATATGTTTATCAATGGTTACTACTTAAATGTGTTTAAAACACAGTCTACTGATGTCAATCGACCTTATCAGGAC
AAGGACAAACGAGGTGGTATTTTCGATGCTGTTTTCACCAGAGGAGATCAGACAACGCTCTTGACAGCTCGTCAT
GATTTAAAAAATAAAGGACTAAATGACATCAGCACCATTATCAAGCAAGAACTGACTGAAGGAAGAGCCCTTGCT
TTATCACATACCTACGCCAATGTTAGCATTAGCCATGTGATTAACTTGTGGGGAGCTGATTTTAATGCTGAAGGA
AACCTTGAGGCCATCTATGTCACAGACTCAGATGCTAATGCGTCTATTGGTATGAAAAAATATTTTGTCGGCATT
AATGCTCATGGACATGTCGCCATTTCTGCCAAGAAAATAGAAGGAGAAAACATTGGCGCTCAAGTATTAGGCTTA
TTTACGCTTTCCAGTGGCAAGGACATTTGGCAGAAACTGAGCGGCGGTGGCCATCATCACCATCACCACTAA

pCART206 (SEQ ID NO: 43)
ATGGACGATTACCAAAGGAATGCTACGGAAGCTTATGCCAAAGAAGTACCACATCAGATCACTTCTGTATGGACC
AAAGGTGTTACACCACCCGAGCAGTTTACTCAAGGTGAAGATGTGATCCATGCGCCATATCTTGCTCATCAAGGC
TGGTACGATATCACCAAGGCCTTCGATGGGAAGGATAATCTCTTGTGTGGCGCAGCAACGGCAGGTAATATGCTG
CATTGGTGGTTTGATCAAAATAAAACAGAGATTGAAGCCTATTTAAGTAAACACCCTGAAAAGCAAAAAATCATT
ATTAACAACCAAGAGCTATTTGATTTGAAAGCTGCTATCGATACCAAGGACAGTCAAACCAATAGTCAGCTTTTT
AATTATTTTAGAGATAAAGCCTTTCCAAATCTATCAGCACGTCAACTCGGGGTTATGCCTGATCTTGTTCTAGAT
ATGTTTATCAATGGTTACTACTTAAATGTGTTTAAAACACAGTCTACTGATGTCAATCGACCTTATCAGGACAAG
GACAAACGAGGTGGTATTTTCGATGCTGTTTTCACCAGAGGAGATCAGACAACGCTCTTGACAGCTCGTCATGAT
TTAAAAAATAAAGGACTAAATGACATCAGCACCATTATCAAGCAAGAACTGACTGAAGGAAGAGCCCTTGCTTTA
TCACATACCTACGCCAATGTTAGCATTAGCCATGTGATTAACTTGTGGGGAGCTGATTTTAATGCTGAAGGAAAC
CTTGAGGCCATCTATGTCACAGACTCAGATGCTAATGCGTCTATTGGTATGAAAAAATATTTTGTCGGCATTAAT
GCTCATGGACATGTCGCCATTTCTGCCAAGAAAATAGAAGGAGAAAACATTGGCGCTCAAGTATTAGGCTTATTT
ACGCTTTCCAGTGGCAAGGACATTTGGCAGAAACTGAGCGGCGGTGGCCATCATCACCATCACCACTAA

pCART207 (SEQ ID NO: 44)
ATGGACGATTACCAAAGGAATGCTACGGAAGCTTATGCCAAAGAAGTACCACATCAGATCACTTCTGTATGGACC
AAAGGTGTTACACCACCCGAGCAGTTTACTCAAGGTGAAGATGTGATCCATGCGCCATATCTTGCTCATCAAGGC
TGGTACGATATCACCAAGGCCTTCGATGGGAAGGATAATCTCTTGTGTGGCGCAGCAACGGCAGGTAATATGCTG
CATTGGTGGTTTGATCAAAATAAAACAGAGATTGAAGCCTATTTAAGTAAACACCCTGAAAAGCAAAAAATCATT
TTTCGTAACCAAGAGCTATTTGATTTGAAAGCTGCTATCGATACCAAGGACAGTCAAACCAATAGTCAGCTTTTT
AATTATTTTAGAGATAAAGCCTTTCCAAATCTATCAGCACGTCAACTCGGGGTTATGCCTGATCTTGTTCTAGAT
ATGTTTATCAATGGTTACTACTTAAATGTGTTTAAAACACAGTCTACTGATGTCAATCGACCTTATCAGGACAAG
GACAAACGAGGTGGTATTTTCGATGCTGTTTTCACCAGAGGAGATCAGACAACGCTCTTGACAGCTCGTCATGAT
TTAAAAAATAAAGGACTAAATGACATCAGCACCATTATCAAGCAAGAACTGACTGAAGGAAGAGCCCTTGCTTTA
TCACATACCTACGCCAATGTTAGCATTAGCCATGTGATTAACTTGTGGGGAGCTGATTTTAATGCTGAAGGAAAC
CTTGAGGCCATCTATGTCACAGACTCAGATGCTAATGCGTCTATTGGTATGAAAAAATATTTTGTCGGCATTAAT
GCTCATGGACATGTCGCCATTTCTGCCAAGAAAATAGAAGGAGAAAACATTGGCGCTCAAGTATTAGGCTTATTT
ACGCTTTCCAGTGGCAAGGACATTTGGCAGAAACTGAGCGGCGGTGGCCATCATCACCATCACCACTAA

pCART208 (SEQ ID NO: 45)
ATGGACGATTACCAAAGGAATGCTACGGAAGCTTATGCCAAAGAAGTACCACATCAGATCACTTCTGTATGGACC
AAAGGTGTTACACCACCCGAGCAGTTTACTCAAGGTGAAGATGTGATCCATGCGCCATATCTTGCTCATCAAGGC
TGGTACGATATCACCAAGGCCTTCGATGGGAAGGATAATCTCTTGTGTGGCGCAGCAACGGCAGGTAATATGCTG
CATTGGTGGTTTGATCAAAATAAAACAGAGATTGAAGCCTATTTAAGTAAACACCCTGAAAAGCAAAAAATCATT
ATTCGTAACCAAGAGCTATTTGATTTGAAAGCTGCTATCGATACCAAGGACAGTCAAACCAATAGTCAGCTTTTT
AATTATTTTAGAGATAAAGCCTTTCCAAATCTATCAGCACGTCAACTCGGGGTTATGCCTGATCTTGTTCTAGAT
ATGTTTATCAATGGTTACTACTTAAATGTGTTTAAAACACAGTCTACTGATGTCAATCGACCTTATCAGGACAAG
GACAAACGAGGTGGTATTTTCGATGCTGTTTTCACCAGAGGAGATCAGACAACGCTCTTGACAGCTCGTCATGAT
TTAAAAAATAAAGGACTAAATGACATCAGCACCATTATCAAGCAAGAACTGACTGAAGGAAGAGCCCTTGCTTTA
TCACATACCTACGCCAATGTTAGCATTAGCCATGTGATTAACTTGTGGGGAGCTGATTTTAATGCTGAAGGAAAC
CTTGAGGCCATCTATGTCACAGACTCAGATGCTAATGCGTCTATTGGTATGAAAAAATATTTTGTCGGCATTAAT
GCTCATGGACATGTCGCCATTTCTGCCAAGAAAATAGAAGGAGAAAACATTGGCGCTCAAGTATTAGGCTTATTT
ACGCTTTCCAGTGGCAAGGACATTTGGCAGAAACTGAGCGGCGGTGGCCATCATCACCATCACCACTAA

pCART210 (SEQ ID NO: 46)
ATGGATGATTATCAGCGCAACGCGACCGAAGCGTATGCGAAAGAAGTGCCGCATCAGATTACCAGCGTGTGGACC
AAAGGCGTGACCCCGCCGGAACAGTTTACTCAAGGTGAAGATGTGATTCATGCGCCGTATCTGGCGAACCAGGGC
TGGTATGATATTACCAAAGCGTTTGATGGCAAAGATAACCTGCTGTGCGGCGCGGCGACCGCGGGCAACATGCTG
CATTGGTGGTTTGATCAGAACAAAACCGAAATTGAAGCGTATCTGAGCAAACATCCGGAAAAACAGAAAATTATT
TTTCGCAACCAGGAACTGTTTGATCTGAAAGAAGCGATTCGCACCAAAGATAGCCAGACCAACAGCCAGCTGTTT
GAATATTTTCGCGATAAAGCGTTTCCGTATCTGAGCGCGCGCCAGCTGGGCGTGATGCCGGATCTGGTGCTGGAT
ATGTTTATTAACGGCTATTATCTGAACGTGTTTAAAACCCAGAGCACCGATGTGAAACGCCCGTATCAGGATAAA

GATAAACGCGGCGGCATTTTTGATGCGGTGTTTACCCGCGGCAACCAGACCACCCTGCTGACCGCGCGCCATGAT
CTGAAAAACAAAGGCCTGAACGATATTAGCACCATTATTAAAGAAGAACTGACCAAAGGCCGCGCGCTGGCGCTG
AGCCATACCTATGCGAACGTGAGCATTAGCCATGTGATTAACCTGTGGGGCGCGGATTTTAACGCGGAAGGCAAC
CTGGAAGCGATTTATGTGACCGATAGCGATGCGAACGCGAGCATTGGCATGAAAAAATATTTTGTGGGCATTAAC
AAACATGGCCATGTGGCGATTAGCGCGAAAAAAATTGAAGGCGAAAACATTGGCGCGCAGGTGCTGGGCCTGTTT
ACCCTGAGCAGCGGCAAAGATATTTGGCAGAAACTGAACGGCGGTGGCCATCATCACCATCACCACTAA

pCART217 (SEQ ID NO: 47)
ATGTCTGTATGGACCAAAGGTGTTACACCACCCGAGCAGTTTACTCAAGGTGAAGATGTGATCCATGCGCCATAT
CTTGCTCATCAAGGCTGGTACGATATCACCAAGGCCTTCGATGGGAAGGATAATCTCTTGTGTGGCGCAGCAACG
GCAGGTAATATGCTGCATTGGTGGTTTGATCAAAATAAAACAGAGATTGAAGCCTATTTAAGTAAACACCCTGAA
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AATAGTCAGCTTTTTAATTATTTTAGAGATAAAGCCTTTCCAAATCTATCAGCACGTCAACTCGGGGTTATGCCT
GATCTTGTTCTAGATATGTTTATCAATGGTTACTACTTAAATGTGTTTAAAACACAGTCTACTGATGTCAATCGA
CCTTATCAGGACAAGGACAAACGAGGTGGTATTTTCGATGCTGTTTTCACCAGAGGAAACCAGACAACGCTCTTG
ACAGCTCGTCATGATTTAAAAAATAAAGGACTAAATGACATCAGCACCATTATCAAGCAAGAACTGACTGAAGGA
AGAGCCCTTGCTTTATCACATACCTACGCCAATGTTAGCATTAGCCATGTGATTAACTTGTGGGGAGCTGATTTT
AATGCTGAAGGAAACCTTGAGGCCATCTATGTCACAGACTCAGATGCTAATGCGTCTATTGGTATGAAAAAATAT
TTTGTCGGCATTAATGCTCATGGACATGTCGCCATTTCTGCCAAGAAAATAGAAGGAGAAAACATTGGCGCTCAA
GTATTAGGCTTATTTACGCTTTCCAGTGGCAAGGACATTTGGCAGAAACTGAGCGGCGGTGGCCATCATCACCAT
CACCACTAA

pCART219 (SEQ ID NO: 48)
ATGGACGATTACCAAAGGAATGCTACGGAAGCTTATGCCAAAGAAGTACCACATCAGATCACTTCTGTATGGACC
AAAGGTGTTACACCACCCGAGCAGTTTACTCAAGGTGAAGATGTGATCCATGCGCCATATCTTGCTCATCAAGGC
TGGTACGATATCACCAAGGCCTTCGATGGGGCGGATAATCTCTTGTGTGGCGCAGCAACGGCAGGTAATATGCTG
CATTGGTGGTTTGATCAAAATAAAACAGAGATTGAAGCCTATTTAAGTAAACACCCTGAAAAGCAAAAAATCATT
TTTCGTAACCAAGAGCTATTTGATTTGAAAGCTGCTATCGATACCAAGGACAGTCAAACCAATAGTCAGCTTTTT
AATTATTTTAGAGATAAAGCCTTTCCAAATCTATCAGCACGTCAACTCGGGGTTATGCCTGATCTTGTTCTAGAT
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GACAAACGAGGTGGTATTTTCGATGCTGTTTTCACCAGAGGAAATCAGACAACGCTCTTGACAGCTCGTCATGAT
TTAAAAAATAAAGGACTAAATGACATCAGCACCATTATCAAGCAAGAACTGACTGAAGGAAGAGCCCTTGCTTTA
TCACATACCTACGCCAATGTTAGCATTAGCCATGTGATTAACTTGTGGGGAGCTGATTTTAATGCTGAAGGAAAC
CTTGAGGCCATCTATGTCACAGACTCAGATGCTAATGCGTCTATTGGTATGAAAAAATATTTTGTCGGCATTAAT
GCTCATGGACATGTCGCCATTTCTGCCAAGAAAATAGAAGGAGAAAACATTGGCGCTCAAGTATTAGGCTTATTT
ACGCTTTCCAGTGGCAAGGACATTTGGCAGAAACTGAGCGGCGGTGGCCATCATCACCATCACCACTAA

pCART226 (SEQ ID NO: 49)
ATGTCTGTATGGACCAAAGGTGTTACACCACCCGAGCAGTTTACTCAAGGTGAAGATGTGATCCATGCGCCATAT
CTTGCTCATCAAGGCTGGTACGATATCACCAAGGCCTTCGATGGGGCGGATAATCTCTTGTGTGGCGCAGCAACG
GCAGGTAATATGCTGCATTGGTGGTTTGATCAAAATAAAACAGAGATTGAAGCCTATTTAAGTAAACACCCTGAA
AAGCAAAAAATCATTTTTCGTAACCAAGAGCTATTTGATTTGAAAGCTGCTATCGATACCAAGGACAGTCAAACC
AATAGTCAGCTTTTTAATTATTTTAGAGATAAAGCCTTTCCAAATCTATCAGCACGTCAACTCGGGGTTATGCCT
GATCTTGTTCTAGATATGTTTATCAATGGTTACTACTTAAATGTGTTTAAAACACAGTCTACTGATGTCAATCGA
CCTTATCAGGACAAGGACAAACGAGGTGGTATTTTCGATGCTGTTTTCACCAGAGGAAATCAGACAACGCTCTTG
ACAGCTCGTCATGATTTAAAAAATAAAGGACTAAATGACATCAGCACCATTATCAAGCAAGAACTGACTGAAGGA
AGAGCCCTTGCTTTATCACATACCTACGCCAATGTTAGCATTAGCCATGTGATTAACTTGTGGGGAGCTGATTTT
AATGCTGAAGGAAACCTTGAGGCCATCTATGTCACAGACTCAGATGCTAATGCGTCTATTGGTATGAAAAAATAT
TTTGTCGGCATTAATGCTCATGGACATGTCGCCATTTCTGCCAAGAAAATAGAAGGAGAAAACATTGGCGCTCAA
GTATTAGGCTTATTTACGCTTTCCAGTGGCAAGGACATTTGGCAGAAACTGAGCGGCGGTGGCCATCATCACCAT
CACCACTAA

pCART229 (SEQ ID NO: 50)
ATGGACGATTACCAAAGGAATGCTACGGAAGCTTATGCCAAAGAAGTACCACATCAGATCACTTCTGTATGGACC
AAAGGTGTTACACCACCCGAGCAGTTTACTCAAGGTGAAGATGTGATCCATGCGCCATATCTTGCTCATCAAGGC
TGGTACGATATCACCAAGGCCTTCGATGGGAAGGATAATCTCTTGTGTGGCGCAGCAACGGCAGGTAATATGCTG
CATTGGTGGTTTGATCAAAATAAAACAGAGATTGAAGCCTATTTAAGTAAACACCCTGAAAAGCAAAAAATCATT
TTTCGTAACCAAGAGCTATTTGATTTGAAAGCTGCTATCGATACCAAGGACAGTCAAACCAATAGTCAGCTTTTT
AATTATTTTAGAGATAAAGCCTTTCCAAATCTATCAGCACGTCAACTCGGGGTTATGCCTGATCTTGTTCTAGAT
ATGTTTATCAATGGTTACTACTTAAATGTGTTTAAAACACAGTCTACTGATGTCAATCGACCTTATCAGGACAAG
GACAAACGAGGTGGTATTTTCGATGCTGTTTTCACCAGAGGAAACCAGACAACGCTCTTGACAGCTCGTCATGAT

TTAAAAAATAAAGGACTAAATGACATCAGCACCATTATCAAGCAAGAACTGACTGAAGGAAGAGCCCTTGCTTTA
TCACATACCTACGCCAATGTTAGCATTAGCCATGTGATTAACTTGTGGGGAGCTGATTTTAATGCTGAAGGAAAC
CTTGAGGCCATCTATGTCACAGACTCAGATGCTAATGCGTCTATTGGTATGAAAAAATATTTTGTCGGCATTAAT
GCTCATGGACATGTCGCCATTTCTGCCAAGAAAATAGAAGGAGAAAACATTGGCGCTCAAGTATTAGGCTTATTT
ACGCTTTCCAGTGGCAAGGACATTTGGCAGAAACTGAGCGGCGGTGGCCATCATCACCATCACCACTAA

pCART191 (SEQ ID NO: 51)
ATGGATGATTATCAGCGCAACGCGACCGAAGCGTATGCGAAAGAAGTGCCGCATCAGATTACCAGCGTGTGGACC
AAAGGCGTGACCCCGCTGACCCCGGAACAGTTTACCCAGGGCGAAGATGTGTTTCATGCGCCGTATGTGGCGAAC
CAGGGCTGGTATGATATTACCAAAGCGTTTGATGGCAAAGATAACCTGCTGTGCGGCGCGGCGACCGCGGGCAAC
ATGCTGCATTGGTGGTTTGATCAGAACAAAGATCAGATTAAACGCTATCTGGAAGAACATCCGGAAAAACAGAAA
ATTAACTTTAACGGCGAAAACATGTTTGATGTGAAAAAAGCGATTGATACCAAAAACCATCAGCTGGATAGCAAA
CTGTTTAACTATTTTAAAGAAAAAGCGTTTCCGTATCTGAGCGCGAAACATCTGGGCGTGTTTCCGGATCATGTG
ATTGATATGTTTATTAACGGCTATCGCCTGAGCCTGACCAACCATGGCCCGACCCCGGTGAAAGAAGGCAGCAAA
GATCCGCGCGGCGGCATTTTTGATGCGGTGTTTACCCGCGGCAACCAGAGCAAACTGCTGACCAGCCGCCATGAT
TTTAAAAACAAAAACCTGAACGATATTAGCACCATTATTAAACAGGAACTGACCAAAGGCAAAGCGCTGGGCCTG
AGCCATACCTATGCGAACGTGCGCATTAACCATGTGATTAACCTGTGGGGCGCGGATTTTAACGCGGAAGGCAAC
CTGGAAGCGATTTATGTGACCGATAGCGATAGCAACGCGAGCATTGGCATGAAAAAATATTTTGTGGGCGTGAAC
GCGCATGGCCATGTGGCGATTAGCGCGAAAAAAATTGAAGGCGAAAACATTGGCGCGCAGGTGCTGGGCCTGTTT
ACCCTGAGCACCGGCCAGGATAGCTGGCAGAAACTGAGCGGCGGTGGCCATCATCACCATCACCACTAA

pCART192 (SEQ ID NO: 52)
ATGGATGATTATCAGCGCAACGCGACCGAAGCGTATGCGAAAGAAGTGCCGCATCAGATTACCAGCGTGTGGACC
AAAGGCGTGACCCCGCTGACCCCGGAACAGTTTACCCAGGGCGAAGATGTGTTTCATGCGCCGTATGTGGCGAAC
CAGGGCTGGTATGATATTACCAAAGCGTTTGATGGCAAAGATAACCTGCTGTGCGGCGCGGCGACCGCGGGCAAC
ATGCTGCATTGGTGGTTTGATCAGAACAAAGATCAGATTAAACGCTATCTGGAAGAACATCCGGAAAAACAGAAA
ATTAACTTTCGCGGCGAAAACATGTTTGATGTGAAAGAAGCGATTCGCACCAAAAACCATCAGCTGGATAGCAAA
CTGTTTGAATATTTTAAAGAAAAAGCGTTTCCGTATCTGAGCGCGAAACATCTGGGCGTGTTTCCGGATCATGTG
ATTGATATGTTTATTAACGGCTATCGCCTGAGCCTGACCAACCATGGCCCGACCCCGGTGAAAAAAGGCAGCAAA
GATCCGCGCGGCGGCATTTTTGATGCGGTGTTTACCCGCGGCAACCAGAGCAAACTGCTGACCAGCCGCCATGAT
TTTAAAAACAAAAACCTGAACGATATTAGCACCATTATTAAAAGCGAACTGACCAACGGCAAAGCGCTGGGCCTG
AGCCATACCTATGCGAACGTGCGCATTAACCATGTGATTAACCTGTGGGGCGCGGATTTTAACGCGGAAGGCAAC
CTGGAAGCGATTTATGTGACCGATAGCGATAGCAACGCGAGCATTGGCATGAAAAAATATTTTGTGGGCGTGAAC
AAACATGGCCATGTGGCGATTAGCGCGAAAAAAATTGAAGGCGAAAACATTGGCGCGCAGGTGCTGGGCCTGTTT
ACCCTGAGCACCGGCCAGGATAGCTGGCAGAAACTGAACGGCGGTGGCCATCATCACCATCACCACTAA

pCART193 (SEQ ID NO: 53)
ATGGATGATTATCAGCGCAACGCGACCGAAGCGTATGCGAAAGAAGTGCCGCATCAGATTACCAGCGTGTGGACC
AAAGGCGTGACCCCGCTGACCCCGGAACAGTTTACCCAGGGCGAAGATGTGTTTCATGCGCCGTATGTGGCGAAC
CAGGGCTGGTATGATATTACCAAAACCTTTAACGGCAAAGATGATCTGCTGTGCGGCGCGGCGACCGCGGGCAAC
ATGCTGCATTGGTGGTTTGATCAGAACAAAGATCAGATTAAACGCTATCTGGAAGAACATCCGGAAAAACAGAAA
ATTAACTTTAACGGCGAACAGATGTTTGATGTGAAAGAAGCGATTGATACCAAAAACCATCAGCTGGATAGCAAA
CTGTTTGAATATTTTAAAGAAAAAGCGTTTCCGTATCTGAGCACCAAACATCTGGGCGTGTTTCCGGATCATGTG
ATTGATATGTTTATTAACGGCTATCGCCTGAGCCTGACCAACCATGGCCCGACCCCGGTGAAAGAAGGCAGCAAA
GATCCGCGCGGCGGCATTTTTGATGCGGTGTTTACCCGCGGCAACCAGAGCAAACTGCTGACCAGCCGCCATGAT
TTTAAAGAAAAAAAACCTGAAAGAAATTAGCGATCTGATTAAACAGGAACTGACCGAAGGCAAAGCGCTGGGCCTG
AGCCATACCTATGCGAACGTGCGCATTAACCATGTGATTAACCTGTGGGGCGCGGATTTTGATGCGGAAGGCAAC
CTGAAAGCGATTTATGTGACCGATAGCGATAGCAACGCGAGCATTGGCATGAAAAAATATTTTGTGGGCGTGAAC
GCGGCGGGCAAAGTGGCGATTAGCGCGAAAAAAATTGAAGGCGAAAACATTGGCGCGCAGGTGCTGGGCCTGTTT
ACCCTGAGCACCGGCCAGGATAGCTGGAACCAGACCAGCGGCGGTGGCCATCATCACCATCACCACTAA

pCART194 (SEQ ID NO: 54)
ATGGATGATTATCAGCGCAACGCGACCGAAGCGTATGCGAAAGAAGTGCCGCATCAGATTACCAGCGTGTGGACC
AAAGGCGTGACCCCGCTGACCCCGGAACAGTTTACCCAGGGCGAAGATGTGTTTCATGCGCCGTATGTGGCGAAC
CAGGGCTGGTATGATATTACCAAAACCTTTAACGGCAAAGATGATCTGCTGTGCGGCGCGGCGACCGCGGGCAAC
ATGCTGCATTGGTGGTTTGATCAGAACAAAGATCAGATTAAACGCTATCTGGAAGAACATCCGGAAAAACAGAAA
ATTAACTTTCGCGGCGAACAGATGTTTGATGTGAAAGAAGCGATTCGCACCAAAAACCATCAGCTGGATAGCAAA
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ATTGATATGTTTATTAACGGCTATCGCCTGAGCCTGACCAACCATGGCCCGACCCCGGTGAAAAAAGGCAGCAAA
GATCCGCGCGGCGGCATTTTTGATGCGGTGTTTACCCGCGGCAACCAGAGCAAACTGCTGACCAGCCGCCATGAT
TTTAAAGAAAAAAAACCTGAAAGAAATTAGCGATCTGATTAAAGAAGAACTGACCAAAGGCAAAGCGCTGGGCCTG

AGCCATACCTATGCGAACGTGCGCATTAACCATGTGATTAACCTGTGGGGCGCGGATTTTGATGCGGAAGGCAAC
CTGAAAGCGATTTATGTGACCGATAGCGATAGCAACGCGAGCATTGGCATGAAAAAATATTTTGTGGGCGTGAAC
AAAGCGGGCAAAGTGGCGATTAGCGCGAAAAAAATTGAAGGCGAAAACATTGGCGCGCAGGTGCTGGGCCTGTTT
ACCCTGAGCACCGGCCAGGATAGCTGGAACCAGACCAACGGCGGTGGCCATCATCACCATCACCACTAA

pCART205 (SEQ ID NO: 55)
ATGGATGATTATCAGCGCAACGCGACCGAAGCGTATGCGAAAGAAGTGCCGCATCAGATTACCAGCGTGTGGACC
AAAGGCGTGACCCCGCCGGAACAGTTTACTCAAGGTGAAGATGTGATTCATGCGCCGTATGTGGCGAACCAGGGC
TGGTATGATATTACCAAAGCGTTTGATGGCAAAGATAACCTGCTGTGCGGCGCGGCGACCGCGGGCAACATGCTG
CATTGGTGGTTTGATCAGAACAAAGATCAGATTAAACGCTATCTGGAAGAACATCCGGAAAAACAGAAAATTAAC
TTTCGCGGCGAACAGATGTTTGATGTGAAAAAAGCGATTGATACCAAAAACCATCAGCTGGATAGCAAACTGTTT
AACTATTTTAAAGAAAAAGCGTTTCCGGGCCTGAGCGCGCGCCGCATTGGCGTGTTTCCGGATCATGTGATTGAT
ATGTTTATTAACGGCTATCGCCTGAGCCTGACCAACCATGGCCCGACCCCGGTGAAAGAAGGCAGCAAAGATCCG
CGCGGCGGCATTTTTGATGCGGTGTTTACCCGCGGCAACCAGAGCAAACTGCTGACCAGCCGCCATGATTTTAAA
AACAAAAACCTGAACGATATTAGCACCATTATTAAACAGGAACTGACCAAAGGCAAAGCGCTGGGCCTGAGCCAT
ACCTATGCGAACGTGAGCATTAACCATGTGATTAACCTGTGGGGCGCGGATTTTAACGCGGAAGGCAACCTGGAA
GCGATTTATGTGACCGATAGCGATAGCAACGCGAGCATTGGCATGAAAAAATATTTTGTGGGCGTGAACGCGCAT
GGCCATGTGGCGATTAGCGCGAAAAAAATTGAAGGCGAAAACATTGGCGCGCAGGTGCTGGGCCTGTTTACCCTG
AGCACCGGCCAGGATAGCTGGCAGAAACTGAGCGGCGGTGGCCATCATCACCATCACCACTAA

Example 2- Assessment of potency (IgG cleavage efficacy)

ELISA

Enzymatic activity was measured using an ELISA-based potency assay. The principle of the ELISA was to coat wells of a multi titre plate with an antibody target (BSA), then incubate different concentrations of IgG cysteine protease polypeptide (test or control) with anti-BSA antibody in the wells, before detecting the quantity of anti-BSA antibody bound to the wells using a detector antibody. The higher the concentration of a given IgG cysteine protease polypeptide in a well, the less intact anti-BSA polypeptide will be bound to the well, giving a lower signal. Similarly, a more potent IgG cysteine protease polypeptide will give a lower signal than a less potent IgG cysteine protease polypeptide when present at the same concentration.

The reference IdeS BX1001865 was prepared as a titration series in 1:2 dilution steps from 320 nM down to 0.16 nM to allow plotting of a standard calibration curve for the assay. The results achieved in the assay for multiple known concentrations of each tested polypeptide were compared against the linear section of the calibration curve to determine the concentration of reference IdeS which achieved the same potency. Dividing the known concentration of each polypeptide by the determined equivalent concentration of reference IdeS from the curve, a score is produced which is the fold change in potency relative to reference IdeS BX1001865. For example, if 5nM test polypeptide achieves a result equivalent to 10nM reference IdeS on the calibration curve, the test polypeptide has a potency 2 fold greater than reference IdeS BX1001865. A mean score for fold change in potency relative to reference IdeS BX1001865 was calculated from all of the scores achieved at the different

concentrations for each tested polypeptide, provided that they fell within the linear section of the calibration curve. This mean score was then compared to the mean score achieved for pCART124 reference IdeS, which was included on each plate to enable comparison between plates. The mean score for pCART124 is divided by the mean score for the test polypeptide to produce a "pCART124 ratio", which is effectively the fold change in potency relative to IdeS for each polypeptide. This pCART124 ratio could then be visualised on a bar diagram.

Briefing summarising the laboratory protocol: Wells of multi-titre plates were coated overnight with BSA (10 μg/ml), then washed with PBS-T and blocked for 1 hour with 2% fish skin gelatine in PBS. IdeS BX1001865 polypeptide was prepared as a titration series in 1:2 dilution steps in PBS with 0.1% gelatine from 320 nM down to 0.16 nM. The test polypeptides and the pCART124 control were then prepared at each of 15, 7.5, 3.75, and 1.9 nM in PBS with 0.1% gelatine. A 50 μl sample of polypeptide was added to each well with 50 μl of rabbit anti-BSA (ACRIS, #R1048P, 10 nM) as substrate. The plates were incubated at room temperature for 1 hour and then washed with PBS-T. Biotinylated goat anti-rabbit Fc-specific antibody (30 000x diluted) was added as a detector antibody and incubated for 30 min. The plate was washed and 40 000x diluted SA-Horseradish Peroxidase (HRP; Pierce) was added and incubated for 30 min. The plates were washed and developed using TMB One Component as a chromogenic substrate for HRP for 7 min, stopped with 0.5 M H_2SO_4. Absorbance (OD) was measured at $\lambda = 450$ nm. Mean scores for fold change in potency relative to BX1001865 were determined for each test polypeptide and for pCART124. The "pCART124 ratio" for each test polypeptide was then calculated as set out above.

The "pCART124 ratio" results for pCART191, 192, 193, 194, 197, 198, 200 and 201 are shown in Figure 1, alongside the result for pCART124. All of the exemplary polypeptides of the invention shown here achieve at least equivalent potency relative to the IdeS control (pCART124). pCART194, 197, 200 and 201 all achieve much higher potency, even as high as 8.0 fold improvement over control for pCART197 and pCART201.

Interestingly pCART200 and 201 both involve modifications to the N terminal end. Also, pCART194 and 197 each have the N138R/K modification. A change to a positive amino acid at the position corresponding to position 139 of SEQ ID NO: 3 is expected to produce similar results to the N138R/K substitution. Positions 138 and 139 are situated in the loop of a beta hairpin structure spanning positions 134 to 144 of SEQ ID NO: 3. Based on the results obtained herein, changes to positive amino acids in either or both of positions 138 and 139 are expected to increase IgG cysteine protease activity.

The results for pCART202, 203 and 204 are shown in Figure 2. pCART203 in particular is around 3.5 fold more potent than IdeS. pCART202 is between 1 and 1.5 fold more potent than IdeS. pCART204 is of comparable potency to pCART144.

Visualisation of IgG cleavage patterns

The efficacy of the different pCART polypeptides was further evaluated by visualising on SDS-PAGE the cleavage products produced by a titration series of each polypeptide in different substrates. To test efficacy in pure IgG substrate, adalimumab (Humira) was used for IgG1 and denosumab (XGEVA) for IgG2. To test efficacy in a more complex physiological environment, some of the polypeptides were also titrated in in IVIg (Octagam). This allows the evaluation of the impact of neutralizing anti-IdeS antibodies on polypeptide activity. Cleavage patterns for each polypeptide are compared with the cleavage patterns of IdeS (BX1001865 and pCART124) in the same substrate. The protocol was follows:

For the pure IgG tests, each test polypeptide or control was diluted in a 1:3 steps titration series from 6.7 µg/ml down to 0.04 ng/ml in PBS with 0.05% BSA as supporting protein. 25 µl of each concentration was transferred to multi titre plates and the cleavage reaction was starting by adding 25 µl of either Humira or XGEVA (2 mg/ml). Thus each starting concentration of polypeptide is diluted 1:2 in the well, giving a titration series of 3.3 µg/ml down to 0.02 ng/ml.

For the IVIg tests, each test polypeptide or control was diluted in a 1:2 steps titration series from 30 µg/ml down to 0.015 ng/ml in PBS with 0.05% BSA as supporting protein. 25 µl of each concentration was transferred to multi titre plates and the cleavage reaction was starting by adding 25 µl of 10 mg/ml IVIg. Thus each starting concentration of polypeptide is diluted 1:2 in the well, giving a titration series of 15 µg/ml down to 0.0075 ng/ml.

The plates were incubated in 37°C for 1.5 hours. The samples were mixed 1:4 in 2X SDS loading buffer and heated at 92°C for 5 min. 10 µl were loaded on a polyacrylamide gel (15-well 4-20% Mini-PROTEAN®TGX™ precast gel (Biorad) which was read according to standard protocols.

Figure 3 shows the cleavage patterns produced with IgG1 substrate for pCART202, 203 and 204 as compared to both IdeS controls (pCART124 and BX1001865) and IdeZ (pCART144). Enzyme concentrations go from 3.33 µg/ml (lane 1) down to 0.02 ng/ml (lane 12) in a 1:3 step dilution series. Intact adalimumab (without enzyme) is shown in lane 13. The

arrows on the right indicate the different cleavage products from IgG. Arrow 1: Intact IgG; arrow 2: scIgG (single cleaved IgG – results from cleavage of first IgG heavy chain); arrow 3: $F(ab')_2$ fragment (results from cleavage of second IgG heavy chain). Vertical lines were added to facilitate the comparison at the 1st IgG heavy chain cleavage, where Intact IgG becomes scIgG (between lane 6 and 7) and at the 2nd IgG heavy chain cleavage, where scIgG becomes $F(ab')_2$ fragment (between lane 2 and 3).

The enzyme IdeZ (pCART144) has lower cleavage efficacy of both the 1st and 2nd IgG heavy chain. IdeS (BX1001865 and pCART124) is about 3 fold more effective (i.e. one titration step) than pCART144 in cleavage of both heavy chains. Cleavage at 1.5 ng/ml (lane 8) for IdeS (BX1001865 and pCART124) equals the pCART144 (IdeZ) cleavage at 4.6 ng/ml (lane 7). BX1001865 and pCART124 show intense scIGg bands (arrow 2) at 4.6 ng/ml (lane 7) whereas pCART144 has only a weak scIgG band (arrow 2) at this concentration (lane 7).

Importantly, both pCART202 and pCART203 show increased potency in cleavage of IgG (lane 7 and lane 3) compared to IdeZ (pCART144), resulting in more intense scIgG bands (arrow 2) and more intense F(ab')2 bands (arrow 3). No increased efficacy is seen for the enzyme pCART204. The efficacy of pCART202 in cleaving the 2nd heavy chain is shown to be about the same as for IdeS (BX1001865 and pCART124) (compare lane 3). pCART202 is less effective than IdeS, but more effective than pCART144 for the 1st IgG heavy chain cleavage (compare lane 7). Enzyme pCART203 possess an even higher efficacy than IdeS in cleavage of primarily the 2nd heavy chain, resulting in a more intense F(ab')2 band (arrow 3) and a weaker scIgG band (arrow 2) compared with BX1001865 and pCART124 (arrow 3 and 2) at 0.37 µg/ml (lane 3).

Thus, overall figure 3 shows that a modifying the IdeZ sequence with the following modifications R70T, Y71del, N72Q, N73G, seen in both pCART202 and pCART203, increases the efficacy of cleavage of the 2nd IgG heavy chain as compared to pCART144 (IdeZ). Introducing in addition the L64_T65del modification also increases the efficacy of cleavage of the 1st heavy chain, seen for pCART203.

Figure 4 shows the cleavage patterns produced with IVIg substrate for pCART202, 203 and 204 as compared to both IdeS controls (pCART124 and BX1001865) and IdeZ (pCART144). Enzyme concentrations go from 30 µg/ml (lane 1) down to 0.015 ng/ml (lane 12) in a 1:2 step dilution series. Intact IVIg (without enzyme) is shown in lane 13, with the exception of the image for pCART203, from which this lane is absent. The arrows on the right indicate the different cleavage products from IgG. Arrow 1: Intact IgG; arrow 2: scIgG (single cleaved IgG – results from cleavage of first IgG heavy chain); arrow 3: $F(ab')_2$ fragment

(results from cleavage of second IgG heavy chain). Vertical lines were added to facilitate the comparison at the 1st IgG heavy chain cleavage, where Intact IgG becomes scIgG (between lane 6 and 7) and at the 2nd IgG heavy chain cleavage, where scIgG becomes F(ab')$_2$ fragment (between lane 2 and 3).

The enzyme pCART144 (IdeZ) shows more effective cleavage at the 1st IgG heavy chain (lane 6) compared to IdeS (BX1001865 and pCART124), resulting in a more intense scIgG band (arrow 2) and a weaker band of intact IgG (arrow 1). This is likely due to a lower level of binding by neutralizing anti-IdeS antibodies to pCART144 (IdeZ) compared to their recognition of IdeS. As seen for pCART202, pCART203 and pCART204 the increased efficacy in 1st heavy chain cleavage is true for all the IdeZ derived enzymes (lane 6). Concentrations of 0.94 ng/ml (lane 6) for pCART202, pCART203 and pCART204 result in an intense band of scIgG (arrow 2), with most of the IgG single cleaved, whereas the same concentration of IdeS results in less than 50% scIgG (lane 6).

However, pCART144 (IdeZ) is worse in cleavage of the 2nd heavy chain compared to IdeS (BX1001865 and pCART124). This results in a more intense scIgG band (arrow 2) from lane 5 (1.9 ng/ml of enzyme) and also in lanes 4 and 3, compared with IdeS for which the cleavage continues to F(ab')2 bands (arrow 3) already at the next titration step (lane 4, 3.75 µg/ml). Notably, pCART203 shows a capacity comparable to IdeS (BX1001865 and pCART124) at the 2nd cleavage site (lane 2, 3 and 4) and a higher cleavage efficacy than both IdeS and IdeZ (pCART144) at the 1st cleavage site (lane 7).

Enzyme pCART203 demonstrates IgG cleavage at 0.5 ng/ml (lane 7) and has generated mainly scIgG (arrow 2) at about 0.9 ng/ml (lane 6). This corresponds to a 2 fold increased efficacy compared to IdeS, which starts the cleavage at 0.9 ng/ml (lane 6) and has a dominating scIgG band at 1.9 ng/ml (lane 5). Overall figure 4 shows that modifying the IdeZ with the modifications L64_T65del, R70T, Y71del, N72Q and N73G increases the efficacy of cleavage of human IgG even in the presence of neutralizing ADA. This is clearly seen in pCART203 as compared to IdeS.

Figure 5 shows the cleavage patterns produced with IgG1 substrate for pCART205, 206, 207, 208 and 210 as compared to both IdeS controls (pCART124 and BX1001865) and IdeZ (pCART144). Enzyme concentrations go from 3.33 µg/ml (lane 1) down to 0.02 ng/ml (lane 12) in a 1:3 step dilution series. The arrows on the right indicate the different cleavage products from IgG. Arrow 1: Intact IgG; arrow 2: scIgG (single cleaved IgG – results from cleavage of first IgG heavy chain); arrow 3: F(ab')$_2$ fragment (results from cleavage of second IgG heavy chain). Vertical lines were added to facilitate the comparison at the 1st IgG

heavy chain cleavage, where Intact IgG becomes scIgG (between lane 6 and 7) and at the 2nd IgG heavy chain cleavage, where scIgG becomes F(ab')$_2$ fragment (between lane 2 and 3).

The enzyme pCART205 shows an increased capacity compared to pCART144 (IdeZ) in cleavage of both IgG heavy chains (lane 6 and 3), resulting in a more intense scIgG band (arrow 2, lane 6) and a very weak band of intact IgG (arrow 1, lane 6) and more intense F(ab')2 band (arrow 3, lane 3) compared to pCART144 (IdeZ) (lane 6 and 3). However, in the absence of neutralising ADA in this experiment (by contrast to that shown in Figure 4), IdeS (pCART124) cleavage activity for pure IgG1 is higher than pCART205.

Polypeptides pCART207 and pCART210 both show increased IgG cleavage efficacy compared to both IdeS (pCART124) and IdeZ (pCART144) (lane 7 for 1st cleavage and lane 3 for 2nd cleavage). The most potent enzyme, pCART207, shows an approximate 3 fold increase of efficacy in cleavage of both IgG heavy chains compared to IdeS (pCART124). Complete conversion to scIgG (arrow 2) for pCART124 is obtained at 14 ng/ml (lane 6) whereas for pCART207 a single scIgG band (arrow 2) is seen already at 4.6 ng/ml (lane 7). A greater increase in efficacy for pCART207 as compared to pCART124 is seen in cleavage of the 2nd heavy chain. A more intense F(ab')2 band (arrow 3) is seen for pCART207 at 41 ng/ml (lane 4) than pCART124 shows at 0.37 µg/ml (lane 3).

pCART207 and pCART210 share the following modifications relative to the IdeZ sequence: L64_T65del, R70T, Y71del, N72Q, N73G, N138R. Thus overall Figure 5 shows that these changes increase the efficacy of cleavage of human IgG1.

Figure 6 shows the cleavage patterns produced with IgG2 substrate for pCART203, 205, 206, 207, 208 and 210 as compared to both IdeS controls (pCART124 and BX1001865) and IdeZ (pCART144). Enzyme concentrations go from 3.33 µg/ml (lane 1) down to 0.02 ng/ml (lane 12) in a 1:3 step dilution series. The arrows on the right indicate the different cleavage products from IgG. Arrow 1: Intact IgG; arrow 2: scIgG (single cleaved IgG – results from cleavage of first IgG heavy chain); arrow 3: F(ab')$_2$ fragment (results from cleavage of second IgG heavy chain). Vertical lines were added to facilitate the comparison at the 1st IgG heavy chain cleavage, where Intact IgG becomes scIgG (between lane 6 and 7) and at the 2nd IgG heavy chain cleavage, where scIgG becomes F(ab')$_2$ fragment (between lane 2 and 3).

Enzymes pCART203 and pCART207 both show an approximate 3 fold increase in cleavage efficacy compared to pCART144 (IdeZ). pCART144 shows a single intense scIgG band (arrow 2) at a concentration of 0.12 µg/ml (lane 4), compared with a dominating scIgG band (arrow 2) for pCART203 and pCART207 at the lower concentration of 41 ng/ml (lane

5). pCART203 and pCART207 are comparable to IdeS (BX1001865 and pCART124) in efficacy of cleavage of both the 1st and 2nd IgG heavy chain (lane 6 and lane 2). However, in the absence of neutralising ADA in this experiment (by contrast to that shown in Figure 4), IdeS (pCART124) cleavage activity is higher than each of pCART206, pCART208 and pCART210 for both heavy chains of pure IgG2. This can be seen from the single intense scIgG band (arrow 2) present even at the highest concentration of enzyme 3.3 μg/ml (lane 1) for pCART206 and pCART208. pCART205 derived from the IdeS/Z hybrid (pCART145) has about the same efficacy as IdeZ (pCART144) in cleavage of pure human IgG2 (lane 5 for 1st cleavage site and lane 2 for 2nd cleavage site), both resulting in a single scIgG band (arrow 2) at 0.12 μg/ml (lane 4) and a dominating F(ab')2 band at the highest concentration 3.3 μg/ml (lane 1).

Overall, figure 6 shows that the best modifications of IdeZ, i.e. which resulted in the highest increase of efficacy in cleaving IgG2, were those found in pCART203 and pCART207. These enzymes share the modifications L64_T65del, R70T, Y71del, N72Q, N73G, with pCART207 additionally possessing the N138R modification.

Figure 7 shows the cleavage patterns produced with IVIg substrate for pCART207, 208 and 210 as compared to IdeS control (BX1001865). Enzyme concentrations go from 30 μg/ml (lane 1) down to 0.015 ng/ml (lane 12) in a 1:2 step dilution series. Intact IVIg (without enzyme) is shown in lane 13. The arrows on the right indicate the different cleavage products from IgG. Arrow 1: Intact IgG; arrow 2: scIgG (single cleaved IgG – results from cleavage of first IgG heavy chain); arrow 3: $F(ab')_2$ fragment (results from cleavage of second IgG heavy chain). Vertical lines were added to facilitate the comparison at the 1st IgG heavy chain cleavage, where Intact IgG becomes scIgG (between lane 6 and 7) and at the 2nd IgG heavy chain cleavage, where scIgG becomes $F(ab')_2$ fragment (between lane 2 and 3).

pCART207, pCART208 and pCART210 all show increased efficacy compared to IdeS (BX1001865) in the cleavage of the 1st IgG heavy chain (lane 6). IdeS (BX1001865) has generated mainly scIgG (arrow 2) at a concentration of 1.9 ng/ml (lane 5). Similar results are obtained at 0.9 ng/ml (lane 6) for both pCART207 and pCART210, and at only 0.5 ng/ml (lane 7) for pCART208. In the case of pCART208 this is approximately a 4 fold increase in cleavage efficacy of the 1st heavy chain. In cleavage of the 2nd heavy chain pCART208 shows an improved cleavage efficacy, resulting in a dominating F(ab')2 band (arrow 3) at 1.9 ng/ml (lane 5), whereas IdeS (BX1001865) has only generated scIgG (arrow 2) at the same concentration.

Overall, Figure 7 shows that pCART207, pCART208 and pCART210 have increased efficacy of cleavage of human IgG in the presence of anti-IdeS neutralizing antibodies (ADA), as compared to IdeS. A similar result was obtained for pCART206 (data not shown). pCART206, 207, 208 and 210 all share the following modifications relative to the IdeZ sequence: L64_T65del, R70T, Y71del, N72Q and N73G. In addition, pCART207, 208 and 210 also share the N138R modification. Thus, Figure 7 also confirms that these different modifications increase the efficacy of cleavage of human IgG in the presence of neutralizing ADA.

Example 3 – Assessment of immunogenicity

Competitive anti-IdeS antibody assay

This assay is based on competition between a test polypeptide and IdeS for binding to anti-IdeS antibody. A pre-incubation of test enzyme and IVIg will enable binding of anti-IdeS antibodies to the tested pCART enzyme. Thereafter the IVIg-enzyme-mix is added to an IdeS-coated plate and any anti-IdeS antibody not bound to test polypeptide will instead bind to the IdeS on the plate. All binding incubations was made in the presence of 2 mM iodoacetic acid (IHAc) to inhibit IgG cleavage and in high salt so that only high affinity binding occurs. After washing, a biotinylated goat anti-human $F(ab')_2$-specific $F(ab')_2$ fragment is used as detector. Poor recognition of test polypeptide by the anti-IdeS antibodies in IVIg will result in high binding of the anti-IdeS antibodies in IVIg to the plate, giving a high signal. Good recognition of test polypeptide by the anti-IdeS antibodies in IVIg will give the opposition result. The detailed protocol is as follows:

Reference IdeS (BX1001865) was coated overnight on multi-titre plates (5 µg/ml), then washed with PBS-T and blocked for 1 hour with 2% BSA in PBS supplemented with 2 mM IHAc and 1 M NaCl. A mixing plate was prepared with stepwise dilutions of test polypeptide and 20 µg/ml IVIg in PBS supplemented with 0.1% BSA, 2 mM IHAc and 1 M NaCl. The mixing plate was incubated for 1 hour at room temperature on a shaker. After incubation, the blocking solution was discarded from the IdeS-coated plate and 50 µl of each mixture from the mixing plate was transferred to the wells of the coated plate. After incubation for 1 hour room temperature on a shaker, the plate was washed with PBS-T and a detector, biotinylated goat anti-human $F(ab')_2$-specific $F(ab')_2$ fragment (20 000x diluted) was added. After incubation for 30 minutes the plate was washed and 40 000x diluted SA-HRP (Pierce) was added and incubated for 30 min. The plate was washed and developed using TMB One Component as a chromogenic substrate for HRP for 7 min, stopped with 0.5

M H_2SO_4. Absorbance (OD) was measured at $\lambda = 450$ nm. The results were inverted (1/OD value) and presented as a ratio compared with pCART124 (1/(test polypeptide/pCART124)) for visualisation in bar diagrams.

The results for pCART191, 192, 193, 194, 197, 198, 200 and 201 are shown in Figure 8. The results for pCART202, 203 and 204 are shown in Figure 9. All of the tested polypeptides show considerable reduction in anti-IdeS antibody recognition as compared to IdeS. The polypeptide showing the least reduction (pCART193) was recognised at a level approximately 60% lower than IdeS. The remaining tested polypeptides were 70 or even 80% lower than IdeS.

Anti-IdeS titre assay

This assay is based on comparing IVIg dilution titres. The different test polypeptide and control IdeS (BX10018865 and pCART124) were coated on micro titre plates. Binding of anti-IdeS antibodies to the test polypeptides or controls was evaluated by adding titrated amounts of IVIg (1:2 step dilution series from 40 to 0.625 µg/ml i.e. in titres corresponding to 1:250 down to 1:16000 diluted serum) to the plates. The dilution buffer is high salt concentration so that only high affinity binding occurs and includes 2 mM IHAc to inhibit IgG cleavage and in high salt so that only high affinity binding occurs. A cut off OD value was set in each experiment to approximately 3 times the blank. The documented result for each tested polypeptide was the dilution titre of IVIg that gave the lowest OD values (lowest binding of anti-IdeS antibody) above the cut off. In other words; less diluted IVIg is needed for polypeptides with low recognition by the anti-IdeS antibodies (ADA) and more diluted IVIg is needed for enzymes which are highly recognized by the ADA. In brief, the protocol was as follows:

Reference IdeS and each test enzyme was coated overnight on multi titre plates (2 µg/ml), washed with PBS-T and blocked for 1 hour with 2% BSA in PBS supplemented with 2 mM IHAc. The blocking solution was discarded from and 50 µl of stepwise dilutions of IVIg (dilution buffer: PBS 1M NaCl + 0.1%BSA + 2 mM IHAc) was added and incubated for 1 hour at room temperature on a shaker. The plates were washed with PBS-T and a detector, biotinylated goat anti-human $F(ab')_2$-specific $F(ab')_2$ fragment (20 000x diluted) was added and incubated for 30 min. The plates were washed and 40 000x diluted SA-HRP (Pierce) was added and incubated for 30 min. The plate was washed and developed using TMB One Component as a chromogenic substrate for HRP for 7 min, stopped with 0.5 M H_2SO_4. Absorbance (OD) was measured at $\lambda = 450$ nm.

The results for pCART202, 203 and 204 are shown in Figure 10. All three tested polypeptides scored 3x dilutions lower than IdeS for recognition by anti-IdeS antibodies.

The results for pCART205, 206, 207, 208 and 210 are shown in Figure 11. All except pCART206 scored 3x dilutions lower than IdeS for recognition by anti-IdeS antibodies. pCART206 scored 2x dilutions lower. Overall, the tested polypeptides are clearly less immunogenic than IdeS.

Summary

The tested polypeptides are generally more effective at cleaving human IgG than IdeZ and/or are at least as effective at cleaving human IgG as IdeS, and are also typically less immunogenic than IdeS.

Example 4 – Assessment of potency

Potency ELISA

To address the cleavage capacity of human IgG1 and IgG2, two ELISA-based potency assays were set up. One assay measuring IgG1 cleavage and the other IgG2 cleavage. EC50 (half maximal effective concentration) values were calculated for the different IgG cysteine protease polypeptides tested. The principle of the assays was to coat wells of a multi titre plate with a $F(ab)_2$-fragment directed to human IgG antibodies with specificity to the Fab region. Then titrated concentrations of IgG cysteine protease polypeptide (test or control) were incubated together with human IgG1 antibody (Humira) or human IgG2 antibody (XGEVA) in the wells. The quantity of intact or single cleaved human IgG (Humira or XGEVA) bound to the wells was measured using a detector antibody directed to human IgG with specificity against the Fc part of the antibody. The higher the concentration of a given IgG cysteine protease polypeptide in a well, the less intact human IgG antibody will be bound to the well, giving a lower signal. Similarly, a more potent IgG cysteine protease polypeptide will give a lower signal than a less potent IgG cysteine protease polypeptide when present at the same concentration. Titration dose-response curves were prepared for the IdeS control (pCART124) and all tested IgG cysteine protease polypeptides, in both the IgG1 (humira) and IgG2 (XGEVA) assay. EC50 values were also calculated for each tested variant, representing the concentration of a polypeptide where 50% of its maximal effect, in the second heavy chain cleavage of the IgG molecule, is observed i.e. the concentration where half of the IgG molecules are single cleaved and half are fully cleaved. A lower EC50 value

represents a more effective IgG cysteine protease. The cleavage of the first IgG heavy chain, IgG to scIgG, is not visible in this assay because the Fc-part of the IgG is still present and can be detected by the Fc specific detector antibody (figure 18).

Brief summary of the laboratory protocol: Wells of multi titre plates were coated overnight (+2-8°C) with Goat-anti-human Fab-specific $F(ab)_2$-fragment (0.5 µg/ml) (Jackson #109-006-097), then washed with PBS+0.05% Tween 20 (PBS-T) and blocked in 0.45% fish gelatin in PBS-T (block buffer) for 45-120 min at room temperature. Control IdeS (pCART124) and the IgG cysteine protease polypeptides to be tested were prepared as titration series in 1:4 dilution steps in block buffer with a starting concentration of 80 µg/ml. Equal volumes (25 µl) of human IgG1 (Humira) at a concentration of 0.5 µg/ml and the titrated amounts of IgG cysteine protease polypeptides were added to the wells and incubated 2 hours with shaking in a controlled temperature environment at 37°C and then washed with PBS-T. Biotinylated mouse anti-human IgG Fc-specific (m-a-hIgG Bio II, Lot: C0013-ZC43C, Southern Biotech) (600 ng/ml) antibody was mixed with Strep-sulfo (200 ng/ml) and added to the multi titre plates. The plates were sealed with aluminum tape and incubated at +25°C for 1 hour with shaking. The plates were then washed in PBS-T and 150 µl of 2x diluted Read buffer T (MSD read buffer T, Cat. no. R92TC-2) were added to each well. The plates were immediately read on a Plate reader, MSD (Meso Scale Discovery) QuickPlex SQ 120 Model 1300.

Efficacy assays visualised on gel: Assay conducted as described in Example 2 for cleavage of IgG1 (Humira), IgG2 (XGEVA) as well as cleavage of a pool of human IgG, IVIg (Octagam).

Results

Potency ELISA

The resulting dose-response curves for the tested IgG cysteine proteases in the potency assays are shown in figure 12 (IgG1 cleavage) and figure 13 (IgG2 cleavage). pCART207, 217, 219 of the exemplary polypeptides of the invention tested here have improved potency (decreased EC50 values) in cleaving both heavy chains of IgG1 (figure 12) compared to the IdeS control pCART124 (table 1), with a fold improvement in potency of 1.4 for pCART219, 3.2 for pCART217 and as much as 4.0 for pCART207. pCART226 shows a somewhat lower potency than IdeS (pCART124) with a fold difference in EC50 of 0.6 (table 1). For cleavage of IgG2 (figure 13) all of the tested polypeptides show a lower potency compared to original IdeS (pCART124), with higher EC50 values (table 1) and a fold

difference below 1, in cleavage of the second IgG heavy chain. However, all of the tested polypeptides are more potent than pCART144 (SEQ ID NO: 27) (data not shown) which is the sequence the IgG cysteine protease polypeptides of the invention are derived from.

Efficacy assays visualised on gel

The cleavage of IgG1 (figure 14A) and IgG2 (figure 14B) visualised on gel clearly show the first and second heavy chain cleavage (the vertical lines in the figures mark the 1st and 2nd IgG heavy chain cleavage by BX1001865 and pCART124 cleavage). The * in the figures illustrate the approximate EC50 value i.e. the concentration where 50% of the IgG is single cleaved (scIgG) and 50% is fully cleaved ($F(ab')_2$). The data from the gels are summarised in table 2 (IgG1 cleavage) and table 3 (IgG2 cleavage). The cleavage of the 1st heavy chain of IgG1 (Humira) is about the same, 1.5 ng/ml for IdeS (pCART124 and BX1001865), pCART207 and 217 but a somewhat higher concentration is needed to get a dominant scIgG band, about 4.6, for pCART219 and 226 (table 2). However, for the 2nd heavy chain cleavage of IgG1 pCART207, 217 and 219 all demonstrate a higher efficacy than IdeS (pCART124 and BX1001864) (table 2), about 3x (one titration step) more effective in cleavage, about 370 ng/ml for IdeS and about 120 ng/ml for pCART207, 217 and 219. In cleavage of IgG2 (XGEVA) (figure 14B) pCART207, 217, 219 all show one titration step (1:3) lower efficacy and pCART226 has about two titration steps (1:6) lower efficacy compared to IdeS in the cleavage of both the 1st and the 2nd heavy chain (table 3). pCART229 shows about the same efficacy as IdeS (BX1001865 and pCART124) in cleavage of both the 1st (4.6 ng/ml) and 2nd (370 ng/ml) IgG heavy chain of IgG1 (Humira) (figure 15A and table 4), whereas cleavage of IgG2 (XGEVA) by pCART229 is about one titration step (1:3) less effective than IdeS in cleavage of both the 1st and 2nd IgG heavy chain (figure 15B and table 4).

The IgG cysteine protease polypeptides pCART207, 217, 219 and 226 were also titrated in the human IgG pool, IVIg (Octagam) with IdeS (BX1001865) as control (Figure 16). All of them showed a higher efficacy in cleavage of the 1st IgG heavy chain of IVIg compared to IdeS. pCART207, 217, 219 and 226 all needed 0.75 µg/ml to achieve the 1st cleavage whereas IdeS (BX1001865) needed 1.5 µg/ml to generate scIgG (figure 16 and table 5). In the 2nd cleavage pCART207 and 217 are both more efficient than IdeS (BX1001865) with a concentration of 3 µg/ml to generate predominantly $F(ab')_2$ fragments and IdeS needs about 6 µg/ml (figure 16 and table 5). pCART219 and 226 are both less effective compared to IdeS in the second cleavage of the IgG pool IVIg. The cleavage of IVIg by pCART229

was analysed in a broader titration spectra with 1:2 dilutions from 30 µg/ml (figure 17) compared to the tested polypeptides in figure 16. The same efficacy is seen for IdeS (BX1001865 and pCART124) and pCART229 (figure 17) with a concentration of 1.9 µg/ml to generate scIgG and 7.5 µg/ml to give $F(ab')_2$ fragments (table 6).

Summary of figures for Example 4

Figure 12. Titration curves for cleavage of IgG1 (Humira) by different IgG cysteine protease polypeptides.

Figure 13. Titration curves for cleavage of IgG2 (XGEVA) by different IgG cysteine protease polypeptides.

Figure 14. IgG cleavage analyzed by SDS-PAGE using titrated (1:3 dilution from 3300 ng/ml) amounts of pCART207, 217, 219 and 226 with BX1001865 and pCART124 (original IdeS) as controls in the same cleavage experiment. A: cleavage of humira (IgG1) and B: cleavage of XGEVA (IgG2). Vertical lines mark the IdeS (BX1001865 and pCART124) concentrations needed to give the 1st and 2nd IgG heavy chain cleavage (where the amount of the cleaved product dominates over the uncleaved product). The * in the figures mark the approximate EC50 value in this experiment.

Figure 15. IgG cleavage analyzed by SDS-PAGE using titrated (1:3 dilution from 3300 ng/ml) amounts of pCART229 with BX1001865 and pCART124 (original IdeS) as controls in the same cleavage experiment. A: cleavage of humira (IgG1) and B: cleavage of XGEVA (IgG2). Vertical lines mark the IdeS (BX1001865 and pCART124) concentrations needed to give the 1st and 2nd IgG heavy chain cleavage (where the amount of the cleaved product dominates over the uncleaved product).

Figure 16. IVIg cleavage analyzed by SDS-PAGE using titrated (1:2 dilution from 6 µg/ml) amounts of the tested IgG cysteine protease polypeptides and IdeS (BX1001865) as control in the same cleavage experiment.

Figure 17. IVIg cleavage analyzed by SDS-PAGE using titrated amounts (1:2 dilution from 30 µg/ml) of pCART229 with BX1001865 and pCART124 (original IdeS) as controls in the same cleavage experiment.

Figure 18. Schematic representation of the cleavage of immunoglobulins by polypeptides of the invention. The enzymatic cleavage of the IgG is performed in two steps. First, one heavy chain of intact IgG is cleaved and single cleaved IgG (scIgG) is generated. Secondly, the next heavy chain is cleaved and the Fc-part is released. The Fc-part is still attached to the Fab-part in the scIgG molecule and since the detector antibody in the potency

ELISA is recognizing the Fc-part of the IgG molecule the assay will not differentiate between complete IgG from scIgG.

Discussion and conclusion

The lower EC50 values of pCART207, 217, 219 in the Humira potency ELISA demonstrate an improved potency in the 2nd cleavage (from scIgG to $F(ab')_2$) of IgG1 compared to pCART124 (original IdeS). The somewhat lower activity of pCART226 in cleavage of IgG1 is shown in both the Humira potency ELISA and in the Humira efficacy assay analysed by SDS-PAGE.

It is demonstrated in the XGEVA potency ELISA that all of the tested polypeptides pCART207, 217, 219 and 226 have a lower potency compared to IdeS (pCART124) in cleavage of both IgG heavy chains of IgG2. However, when visualising the cleavage on gel instead it is clear that pCART207 has about the same activity as IdeS (BX1001865 and pCART124) in the 1st IgG heavy chain cleavage, whereas it is about 3 times less effective (one titration step) in the 2nd cleavage compared to IdeS. The same pattern is seen for pCART229 with a high efficacy in cleavage of IgG1, comparable to the activity of IdeS, but with a lower efficacy for cleavage of IgG2, primarily the cutting of the 2nd IgG heavy chain. By analysing the IgG cleavage on gel the cutting of the 1st heavy chain (from IgG to scIgG) becomes evident, this cleavage is invisible in the potency ELISA using an Fc-specific detector antibody. Most Fc-mediated actions of IgG are lost already in a single cleaved molecule (data not shown), which is central in a clinical situation where the main focus is to incapacitate pathogenic IgG molecules.

IVIg is a pool of human IgG containing approximately 65-70% IgG1, 35-30% IgG2 and IgG3/IgG4 sharing about 1%. Human IVIg also naturally contains anti-IdeS antibodies, from the IgG donor's earlier exposure to *S. pyogenes*, some of these antibodies will be neutralizing i.e. binding of these IdeS specific antibodies to IdeS will diminish or completely demolish the IdeS IgG protease activity. The results of IVIg cleavage by the different IgG cysteine protease polypeptides thereby display the overall cleavage of all four human IgG subclasses in about their normal ratio in human serum but also in the presence of the neutralizing anti-IdeS antibodies.

In general all IgG cysteine protease polypeptides tested have a lower efficacy in IgG2 cleavage compared to IgG1. pCART207, 217 and 219 are more efficient than IdeS in cleaving IgG1 but less efficient in cleaving primarily the 2nd heavy chain of IgG2. The scIgG bands seen in figure 16 in the highest dose (6 μg/ml) of pCART207, 217, 219 and 226 and in

figure 17 for pCART229 are most likely representing the IgG2 molecules in the IgG pool, IVIg (compare figure 14A with 14B and 15A with 15B).

Table 1. EC50 (ng/ml) measured by potency ELISA and fold difference in potency compared to original IdeS (pCART124).

	EC50 (ng/ml) in cleavage of IgG1 (Humira)	Fold improvement in potency	EC50 (ng/ml) in cleavage of IgG2 (XGEVA)	Fold improvement in potency
pCART124	258	1	225	1
pCART207	64	4.0	444	0.5
pCART217	82	3.2	486	0.5
pCART219	183	1.4	1508	0.15
pCART226	433	0.6	3156	0.07

Table 2. Data for IgG1 (Humira) cleavage shown on gel (figure 14A). Concentration (ng/ml) of polypeptide needed to achieve 1st and 2nd IgG cleavage, where the cleaved product dominates in amounts over the uncleaved. Approximate EC50 value (* in figure 14A).

ID	**1st IgG heavy chain IgG to scIgG** Conc. of enzyme (ng/ml)	**2nd IgG heavy chain scIgG to F(ab')2** Conc. of enzyme (ng/ml)	**Approximate EC50 value, i.e. equal amounts of scIgG and F(ab')2 (*)** Conc. of enzyme (ng/ml)
BX1001865	1.5	370	100
pCART124	1.5	370	100
pCART207	1.5	120	10-40
pCART217	1.5	120	40-100
pCART219	4.5	120	40-100
pCART226	4.5	370	100-400

Table 3. Data for IgG2 (XGEVA) cleavage shown on gel (figure 14B). Concentration (ng/ml) of polypeptide needed to achieve 1st and 2nd IgG cleavage, where the cleaved product dominates in amounts over the uncleaved. Approximate EC50 value (* in figure 14B).

ID	**1st IgG heavy chain IgG to scIgG** Conc. of enzyme (ng/ml)	**2nd IgG heavy chain scIgG to F(ab')2** Conc. of enzyme (ng/ml)	**Approximate EC50 value, i.e. equal amounts of scIgG and F(ab')2 (*)** Conc. of enzyme (ng/ml)
BX1001865	14	370	100-400
pCART124	14	370	100-400
pCART207	41	1100	400-1100
pCART217	41	1100	400-1100
pCART219	41	3300	1100
pCART226	120	3300	1100-3300

Table 4. Data for IgG1 (Humira) cleavage and IgG2 (XGEVA) by pCART229 shown on gel (figure 15). Concentration (ng/ml) of polypeptide needed to achieve 1st and 2nd IgG cleavage (where the cleaved product dominates in amounts over the uncleaved).

ID	1st IgG1 (Humira) heavy chain IgG to scIgG Conc. of enzyme (ng/ml)	2nd IgG1 (Humira) heavy chain scIgG to F(ab')2 Conc. of enzyme (ng/ml)	1st IgG2 (XGEVA) heavy chain IgG to scIgG Conc. of enzyme (ng/ml)	2nd IgG2 (XGEVA) heavy chain scIgG to F(ab')2 Conc. of enzyme (ng/ml)
BX1001865	4.6	370	14	1100
pCART124	4.6	370	14	1100
pCART229	4.6	370	122	3300

Table 5. Data for IVIg cleavage by pCART207, 217, 219 and 226 shown on gel (figure 16). Concentration (ng/ml) of polypeptide needed to achieve 1st and 2nd IgG cleavage, where the cleaved product dominates in amounts over the uncleaved.

ID	1st IgG heavy chain IgG to scIgG Conc. of enzyme (ng/ml)	2nd IgG heavy chain scIgG to F(ab')2 Conc. of enzyme (ng/ml)
BX1001865	1500	6000
pCART124	1500	6000
pCART207	750	3000
pCART217	750	3000
pCART219	750	6000
pCART226	750	6000

Table 6. Data for IVIg cleavage by pCART229 shown on gel (figure 17). Concentration (ng/ml) of polypeptide needed to achieve 1st and 2nd IgG cleavage, where the cleaved product dominates in amounts over the uncleaved.

ID	1st IgG heavy chain IgG to scIgG Conc. of enzyme (ng/ml)	2nd IgG heavy chain scIgG to F(ab')2 Conc. of enzyme (ng/ml)
BX1001865	1900	7500
pCART124	1900	7500
pCART229	1900	7500

Example 5 - ADA ELISA, a competitive ELISA for ADA-IdeS binding sites.

Anti-drug antibody (ADA) binding sites against IdeS was measured for "ADA" modified polypeptide of the invention (pCART207, 217, 219 and 226), using an ELISA, Meso Scale Discovery (MSD), based assay. The principle of the ELISA was to coat wells of a multi titre plate with original his-tagged-IdeS (pCART124). Most humans have antibodies against IdeS in their serum due to earlier infections of *S. pyogenes*. Here two different clinical human serum pools were used as standards for detection of ADA. The first pool is normal human serum from a serum pool of 100 individuals, called Human serum pool 1191807, and the second is a pool of serum from patients in the phase II study 13-HMedIdeS-02, called Phase II pool-2. These patients have been administered with IdeS once in a dose-range of 0.24 -0.5 mg/Kg body weight and thereby have induced levels (approximately 50 times) of anti-IdeS ADA in their serum.

The outline of this competitive ADA ELISA is that IdeS (pCART124) is coated in the bottom of a micro titre plate. Human serum pools are pre-incubated together with the polypetide to be tested for ADA recognition sites, or with the positive control IdeS (pCART124) in a molar ration of 1:100 with 100x excess of the tested polypeptide. The concentration of the two different serum pools used for pre-incubation is estimated from the standard curve to give approximately 80% binding to original IdeS. If the ADA binding sites have been abolished in the polypeptides tested, these polypeptides could not compete with the binding of ADA to the original IdeS at the bottom of the wells, i.e. a low signal demonstrates strong ADA-resemblance to the original IdeS (pCART124) and a high signal demonstrates weak ADA-resemblance to the original IdeS.

The concentration of both standards achieving approximately 80% binding at the linear section of the standard curve was about 200 ng ADA (IdeS)/ml. In the competitive pre-incubation this concentration of both standards were used separately and the concentration of the IgG cysteine protease polypeptides were used in a ratio of 100 times the ADA concentration, including the molar weight difference between an antibody of 150 kDa and IdeZ of approximately 35 kDa, 4.2 times, giving 100 times 200 ng/ml dividing with 4.2 giving approx. 5 µg/ml of the tested polypeptides. The standard serum containing 200 ng/ml ADA and the IdeS (pCART124) or tested polypeptides are pre-incubated together for 1 hour at room temperature (RT). As a control for maximum ADA binding, the same concentration of the standards were pre-incubated without IdeS (pCART124) or any other IgG cysteine proteases and used as 80% binding-max value. The lowest level of the standards curve, were

used as lower limit values for the range of the calculation of the competition. The mean score for the standards pre-incubated with IdeS (pCART124) or the tested polypeptides were subtracted with the 80% standard binding value divided with 80% standard binding value subtracted with the lower limit values giving % competition value. The IgG cysteine protease polypeptide with the lowest % competition means that the most ADA binding epitopes have been abolished compared to original IdeS (pCART124).

Brief summary of the laboratory protocol: Wells of multi titre plates were coated overnight with pCART124 (1 µg/ml), washed 3 times with PBS-T and blocked for 1 hour with 0.45% fish skin gelatine and 2 mM of the cysteine protease inhibitor Iodoacetic acid (IHAc) in PBS.

Both standards were prepared as a titration series in 1:3 dilution steps in 0.45% fish skin gelatine and 2 mM IHAc in PBS, from 5000 ng ADA (IdeS)/ml to 2.5 ng ADA (IdeS)/ml to allow plotting of a standard calibration curve for the assay, with measurements at both the linear part and the maximum and minimum part of the standard curve. At the same time as the blocking of the plate, the standards and the IdeS (pCART124) or tested polypeptides were pre-incubated together for 1 hour at RT, i.e. the samples in a competition step, using 200 ng/ml ADA (standards) and 5 µg/ml IdeS control (pCART124) or IgG cysteine protease polypeptides to be tested.

The pCART124 coated plate was washed 3 times and then 50 µl pre-incubated samples or 50 µl standard were added to each well of the multi titre plate.

The plate was incubated at RT for 2 hours and then washed with PBS-T. Goat-anti-human F(ab) specific $F(ab)_2$ fragment-bio (Jackson #109-066-097, 0.65 mg/ml), (1000x diluted) was added as detector antibody and Streptavidin-Sulfo (MSD Cat. No: R32AD-1 or R32AD-5) (2000x diluted) in blocking buffer incubated for 1 hour at RT in the dark. The plate was washed 3 times and Read buffer T (MSD Read buffer T (4x) 4x diluted was added and the plate was analysed on a Plate reader, MSD (Meso Scale Discovery) QuickPlex SQ 120 Model 1300 directly.

Results and discussion

Percentage (%) blocking of IdeS-ADA binding sites for pCART207, 217, 219 and 226 are shown in Figure 19 and Figure 20 and the original IdeS pCART124 is used as positive control for 100% resemblance.

All tested IgG cysteine protease polypeptides, pCART207, 217, 219 and 226 occupy fewer ADA binding sites in human serum compared to original IdeS (pCART124). Patients that

have been treated with IdeS once (Phase II pool-2) have developed more IdeS specific ADA and there were minimal recognition of pCART207, 217 and 219 (Figure 20) compared to the serum pool from healthy volunteers (Human serum pool 1191807) (Figure 19).

Example 6 – Assessment of *in vivo* efficacy in an Octagam (human IVIg) mouse model

In the present study BALB/c mice were injected intraperitoneally (i.p.) with human IVIg (Octagam). The concentration of human IVIg was administered at a dose of 900 mg/kg, to correlate to the human IgG plasma concentration (10 mg/ml).

Human IVIg was injected i.p. day 0. Twenty four hours (day 1) after the injection of human IVIg, PBS, IdeS controls (BX1001865 and pCART124), or the IgG proteases to be tested, pCART207, pCART217, pCART219 and pCART226, were administered intravenously (i.v.) at a dose of 1 mg/kg. Two hours later serum samples were collected and mice were sacrificed.

Efficacy ELISA

The principle of the assay was to coat wells of a multi titre plate with a $F(ab')_2$-fragment directed to human IgG antibodies with specificity to the Fab region. Then serum from mice treated with IVIg and IdeS controls (BX1001865 and pCART124) or the tested IgG cysteine protease polypeptide were added. The quantity of intact or single cleaved human IgG (IVIg) bound to the wells was measured using a detector antibody directed at human IgG (IVIg) with specificity against the Fc part of the antibody. The lower the detected concentration of intact human IgG antibody (IVIg) the more effective the IgG cysteine protease polypeptide is expected to be.

Brief summary of the laboratory protocol: Wells of a multi titre plate were coated overnight (+2-8°C) with Goat-anti-human Fab-specific $F(ab)_2$-fragment (0.5 µg/ml) (Jackson #109-006-097), then washed with PBS+0.05% Tween 20 (PBS-T) and blocked in 2% BSA in PBS-T (block buffer) for 45-120 min at RT (room temperature). The Human Serum Protein Calibrator (DAKO #X0908) was used as a standard and added in a range from 0.5-300 ng/ml. The serum samples taken from mice treated with IVIg and different IgG cysteine protease polypeptides were thawed and diluted in block buffer 100 000 times before addition to the assay multi titre plate. The plate was incubated 2 hours with shaking at RT and then washed with PBS-T. Biotinylated mouse anti-human IgG Fc-specific (600 ng/ml) (Jackson #109-066-098) antibody was mixed with Strep-sulfo (200 ng/ml) (MSD #R32AD-1) and added to the

multi titre plate. The plate was sealed with aluminum tape and incubated at RT for 1 hour with shaking. The plate was then washed in PBS-T and 150 µl of 2x diluted Read buffer T (MSD #R92TC-2) was added to each well. The plate was immediately analysed on a plate reader, MSD (Meso Scale Discovery) QuickPlex SQ 120 Model 1300 directly.

Efficacy visualized on gel

To visualize the human IgG cleavage *in vivo* in mouse 10 µl serum was diluted 1:10 in 90 µl PBS. Thereafter 10 µl diluted serum was mixed with 30 µl 4x SDS-PAGE loading buffer. 5 µl of IgG in-house marker was used to show the different IgG fragments (IgG, scIgG and F(ab')2). Samples were heated at 92°C for 3 min (Thermo mixer compact, eppendorf) and briefly centrifuged before loading 10 µl on 4-20% Mini-Protean® TGX, Stain-free™ gel (Cat. #456-8096, Biorad). Gels were run at 200 V for 40 min.

Results and conclusion

In vivo cleavage of human IVIg (Octagam) by IdeS (BX1001865 and pCART124) and pCART207, 217, 219 and 226 were compared by studying the level of human IgG in serum by efficacy ELISA and by analysing the degradation of IgG by SDS-PAGE.

Treatment with IdeS (BX1001865 and pCART 124) and the different IgG cysteine proteases pCART207, pCART217, pCART219 and pCART226 in IVIg-mice clearly demonstrated cleavage of human IgG *in vivo* in this mouse model (Table 7 and figure 21).

Complete cleavage were shown for the IdeS control (BX1001865), pCART207 and pCART217, with no scIgG bands visible and significant $F(ab')_2$ bands on the gels (Figure 22). pCART219 and pCART226 showed a lower efficacy in this mouse model with scIgG molecules still present in the mouse serum after two hours (Figure 22C). However, no intact IVIg could be detected on the gel indicating that the higher concentration of IgG-Fc by the detector antibody (higher bar) for pCART219 and pCART226 in figure 21 comes from scIgG and not intact IgG. Mouse no: 2 in the pCART207 group and mouse no: 4 in the pCART219 group did not receive the IVIg injection (Figure 22B and C), therefor no IgG cleavage fragments were visible on the gel from these animals. The protein band patterns will represent the background proteins in BALB/c mouse serum. This shows that polypeptides of the invention cleave IgG in an *in vivo* model.

Table 7. Analysis of *in vivo* cleavage of human IgG in serum from mice treated with IdeS (BX1001865 and pCART124 / the tested IgG cysteine proteases by the efficacy ELISA (average ± Stdev).

	Average (mg/mL)	Stdev
Control (PBS)	6.58	0.80
BX1001865	0.30	0.05
pCART124	0.39	0.12
pCART207	0.66	0.03
pCART217	0.78	0.05
pCART219	0.99	0.18
pCART226	1.80	0.85

CLAIMS

1. A polypeptide having IgG cysteine protease activity and comprising a variant of the sequence of SEQ ID NO:4 or 5, which variant:

(a) is at least 80% identical to SEQ ID NO: 4 or 5;

(b) has a cysteine (C) at the position in said variant sequence which corresponds to position 102 of SEQ ID NO: 3; and

(c) has, at the positions in said variant sequence which correspond to positions 92, 272, 294 and 296 of SEQ ID NO: 3, a lysine (K), a histidine (H), an aspartic acid (D) and an aspartic acid (D), respectively;

wherein said polypeptide is more effective at cleaving human IgG than IdeZ or is at least as effective at cleaving human IgG as IdeS; and

wherein said variant of the sequence of SEQ ID NO: 4 or 5:

(1) has a positively charged amino acid at the position in said variant which corresponds to position 138 of SEQ ID NO: 3;

(2) has a positively charged amino acid at the position in said variant which corresponds to position 139 of SEQ ID NO: 3;

(3) does not include the contiguous sequence DDYQRNATEA YAKEVPHQIT; or

(4) has at least one of the following modifications:

i. a deletion of the leucine (L) and threonine (T) residues at the positions in said variant which correspond to positions 64 and 65 of SEQ ID NO: 3;

ii. a threonine (T) in place of the arginine (R) at the position in said variant which corresponds to position 70 of SEQ ID NO: 3;

iii. a deletion of the tyrosine (Y) at the position in said variant which corresponds to position 71 of SEQ ID NO: 3;

iv. a glutamine (Q) in place of the asparagine (N) at the position in said variant which corresponds to position 72 of SEQ ID NO: 3;

v. a glycine (G) in place of the asparagine (N) at the position in said variant which corresponds to position 73 of SEQ ID NO: 3;

vi. a alanine (A) in place of the glutamic acid (E) at the position in said variant which corresponds to position 67 of SEQ ID NO: 3; or

vii. a asparagine (N) in place of the glutamine (Q) at the position in said variant which corresponds to position 68 of SEQ ID NO: 3.

Date Reçue/Date Received 2021-02-08

2. The polypeptide according to claim 1, wherein the positively charged amino acid at the position in said variant which corresponds to position 138 of SEQ ID NO: 3 is arginine (R) or lysine (K).

3. The polypeptide according to claim 1, wherein the positively charged amino acid at the position in said variant which corresponds to position 139 of SEQ ID NO: 3 is arginine (R) or lysine (K).

4. The polypeptide according to claim 1, wherein said variant of the sequence of SEQ ID NO: 4 or 5 is at least 90%, 95% or 99% identical to SEQ ID NO: 4 or 5, respectively.

5. The polypeptide according to claim 1, wherein said polypeptide is less immunogenic than IdeS when measured in the same assay.

6. The polypeptide according to claim 1, wherein said polypeptide is no more immunogenic than IdeZ or IdeS/Z, when measured in the same assay.

7. The polypeptide according to any one of claims 1 to 6, which comprises or consists of the sequence of any one of SEQ ID NOs: 6 to 25.

8. The polypeptide according to claim 7, wherein said sequence includes an additional methionine at the N terminus.

9. The polypeptide according to claim 7 or 8, wherein said sequence includes an additional a histidine tag at the C terminus.

10. The polypeptide according to any one of claims 1 to 9, wherein said polypeptide is at least 2.0 fold more effective than IdeZ at cleaving human IgG, when measured in the same assay.

11. The polypeptide according to any one of claims 1 to 10 which is less immunogenic than IdeS when measured in the same assay.

Date Reçue/Date Received 2021-02-08

12. The polypeptide according to claim 11, wherein the immunogenicity of said polypeptide is no more than 85% of the immunogenicity of IdeS when measured in the same assay.

13. A polynucleotide or expression vector which comprises a nucleic acid sequence encoding a polypeptide of any one of claims 1 to 12.

14. A host cell comprising the polynucleotide or expression vector of claim 13.

15. The host cell of claim 14, which is a bacterial cell.

16. The host cell of claim 15, wherein the bacterial cell is a cell of *E. coli.*

17. A composition comprising a polypeptide according to any one of claims 1 to 6 and at least one pharmaceutically acceptable carrier or diluent.

18. A polypeptide according to any one of claims 1 to 6 for the treatment of a disease or condition mediated in whole or in part by pathogenic IgG antibodies.

19. The polypeptide according to claim 18, wherein said disease or condition is Addison's disease, Anti-GBM glomerulonephritis, Anti-neutrophil cytoplasmic antibody-associated vasculitides, Anti-NMDAR Encephalitis, Anti-phospholipid antibody syndrome, Catastrophic APS, Autoimmune bullous skin diseases, Pemphigus foliaceus, fogo selvagem, pemphigus vulgaris, Autoimmune hemolytic anemia, Autoimmune hepatitis, Autoimmune neutropenia, Bullous pemphigoid, Celiac disease, Chronic utricaria, Complete congenital heart block, Diabetes type 1A, Epidermolysis bullosa acquisita, Essential mixed cryoglobulinemia, Goodpasture's syndrome, Graves'disease, Goitre, hyperthyroidism, infiltrative exophthalmos, infiltarative dermopathy. Guillain-Barré syndrome, Acute inflammatory demyelinating polyneuropathy, acute motor axonal neuropathy, Hemophilia - Acquired FVIII deficiency, Idiopathic thrombocytopenic purpura, Lambert-Eaton myasthenic syndrome, Mixed Connective Tissue Disease, Multiple Myeloma, Myasthenia gravis, Myasthenic crisis, Myocarditis, dilated cardiomyopathy, Neuromyelitis Optica, Primary biliary cirrhosis, Primary Progressive Multiple Sclerosis, Rheumatic heart disease, Rheumatoid Arthritis, Serum-sickness, immune complex hypersensitivity, Sjögren Syndrome, Systemic Lupus

Date Reçue/Date Received 2021-02-08

Erythematosus, Lupus nephritis, Stiff-person syndrome, Systemic sclerosis, Transplant rejection or Thrombotic Thrombocytopenic Purpura (TTP).

20. Use of a polypeptide according to any one of claims 1 to 6 for the treatment of a disease or condition mediated in whole or in part by pathogenic IgG antibodies.

21. Use of a polypeptide according to any one of claims 1 to 6 for the manufacture of a medicament for the treatment of a disease or condition mediated in whole or in part by pathogenic IgG antibodies.

22. The use according to claim 20 or 21, wherein said disease or condition is Addison's disease, Anti-GBM glomerulonephritis, Anti-neutrophil cytoplasmic antibody-associated vasculitides, Anti-NMDAR Encephalitis, Anti-phospholipid antibody syndrome, Catastrophic APS, Autoimmune bullous skin diseases, Pemphigus foliaceus, fogo selvagem, pemphigus vulgaris, Autoimmune hemolytic anemia, Autoimmune hepatitis, Autoimmune neutropenia, Bullous pemphigoid, Celiac disease, Chronic utricaria, Complete congenital heart block, Diabetes type 1A, Epidermolysis bullosa acquisita, Essential mixed cryoglobulinemia, Goodpasture's syndrome, Graves'disease, Goitre, hyperthyroidism, infiltrative exophthalmos, infiltarative dermopathy. Guillain-Barré syndrome, Acute inflammatory demyelinating polyneuropathy, acute motor axonal neuropathy, Hemophilia - Acquired FVIII deficiency, Idiopathic thrombocytopenic purpura, Lambert-Eaton myasthenic syndrome, Mixed Connective Tissue Disease, Multiple Myeloma, Myasthenia gravis, Myasthenic crisis, Myocarditis, dilated cardiomyopathy, Neuromyelitis Optica, Primary biliary cirrhosis, Primary Progressive Multiple Sclerosis, Rheumatic heart disease, Rheumatoid Arthritis, Serum-sickness, immune complex hypersensitivity, Sjögren Syndrome, Systemic Lupus Erythematosus, Lupus nephritis, Stiff-person syndrome, Systemic sclerosis, Transplant rejection or Thrombotic Thrombocytopenic Purpura (TTP).

23. An *ex vivo* method for the cleavage of IgG, the method comprising contacting a sample containing IgG with a polypeptide according to any one of claims 1 to 6 under conditions which permit IgG cysteine protease activity to occur.

24. The method according to claim 23 which is conducted to generate Fc and Fab fragments.

Date Reçue/Date Received 2021-02-08

25. The method according to claim 23 or 24, wherein the sample is a blood sample from from a subject suffering from a disease or condition as defined in claim 19 or 22.

Date Reçue/Date Received 2021-02-08

FIGURE 1

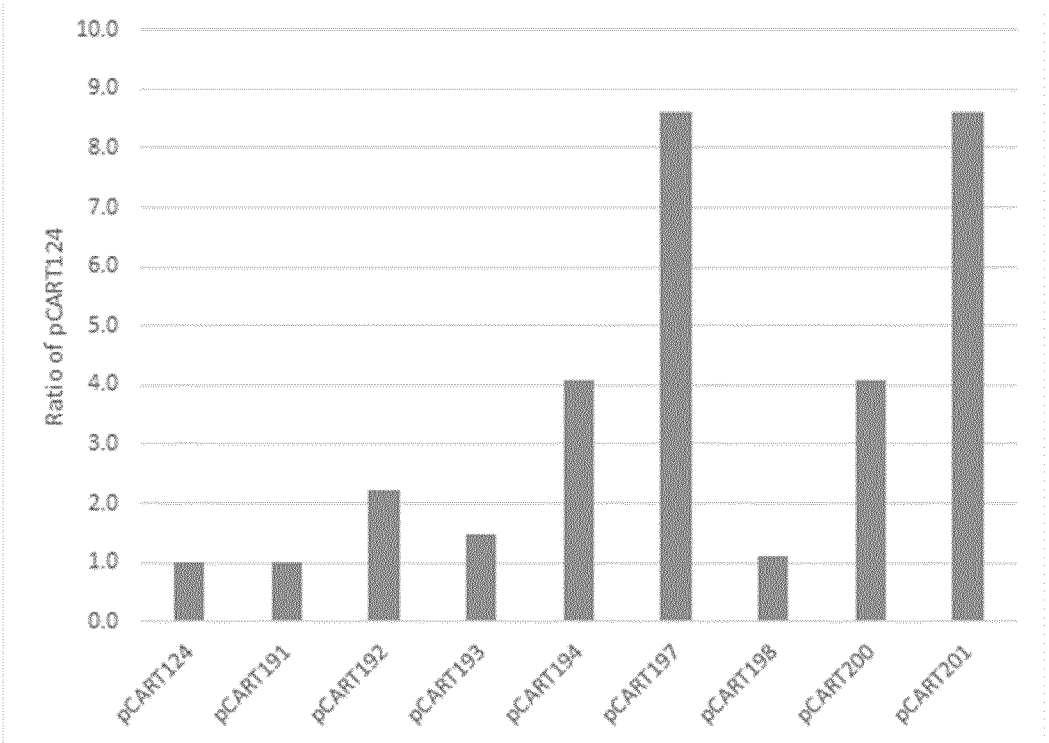


FIGURE 2

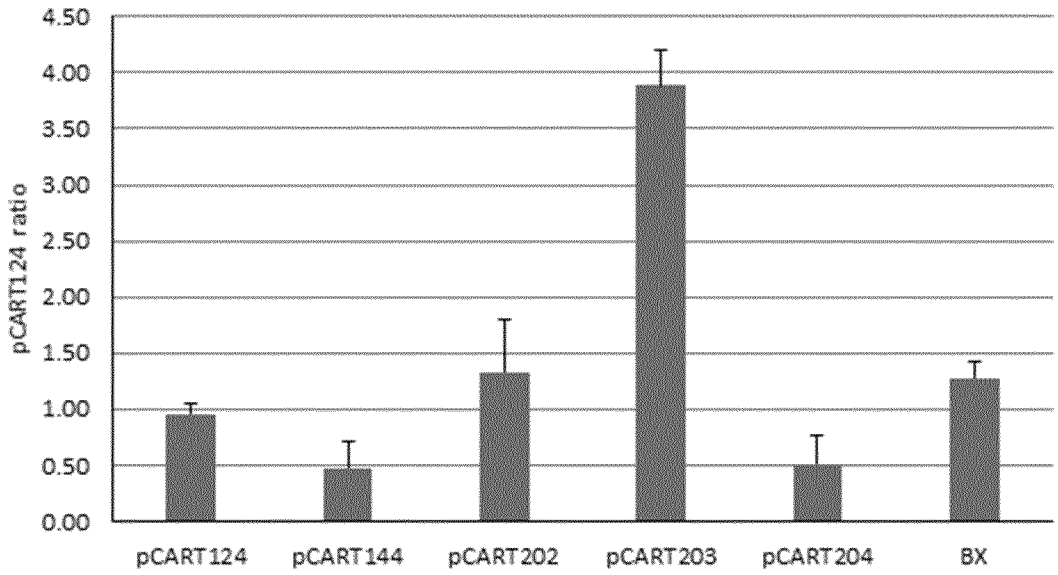
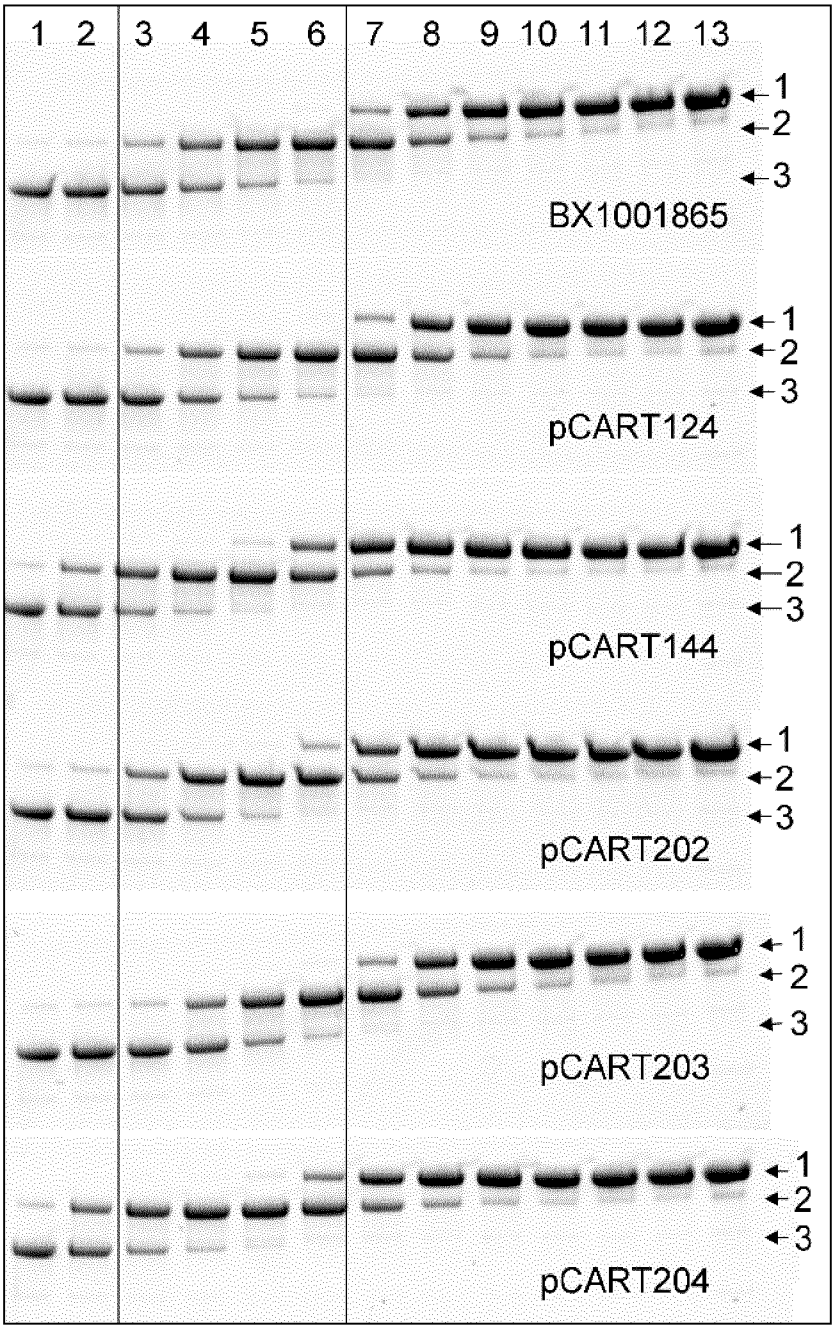


FIGURE 3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2
3
pCART203
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2
3
pCART204

FIGURE 4

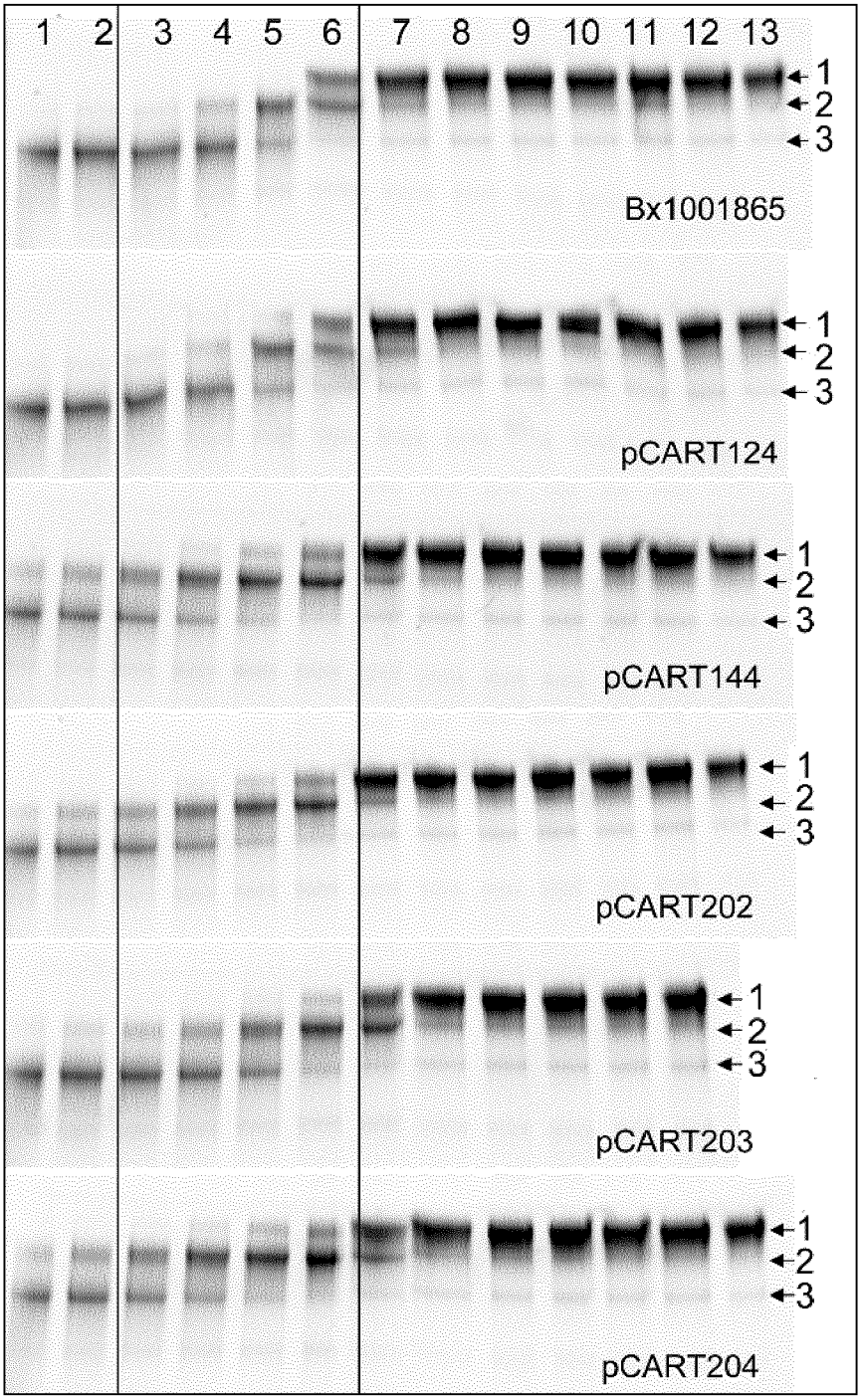
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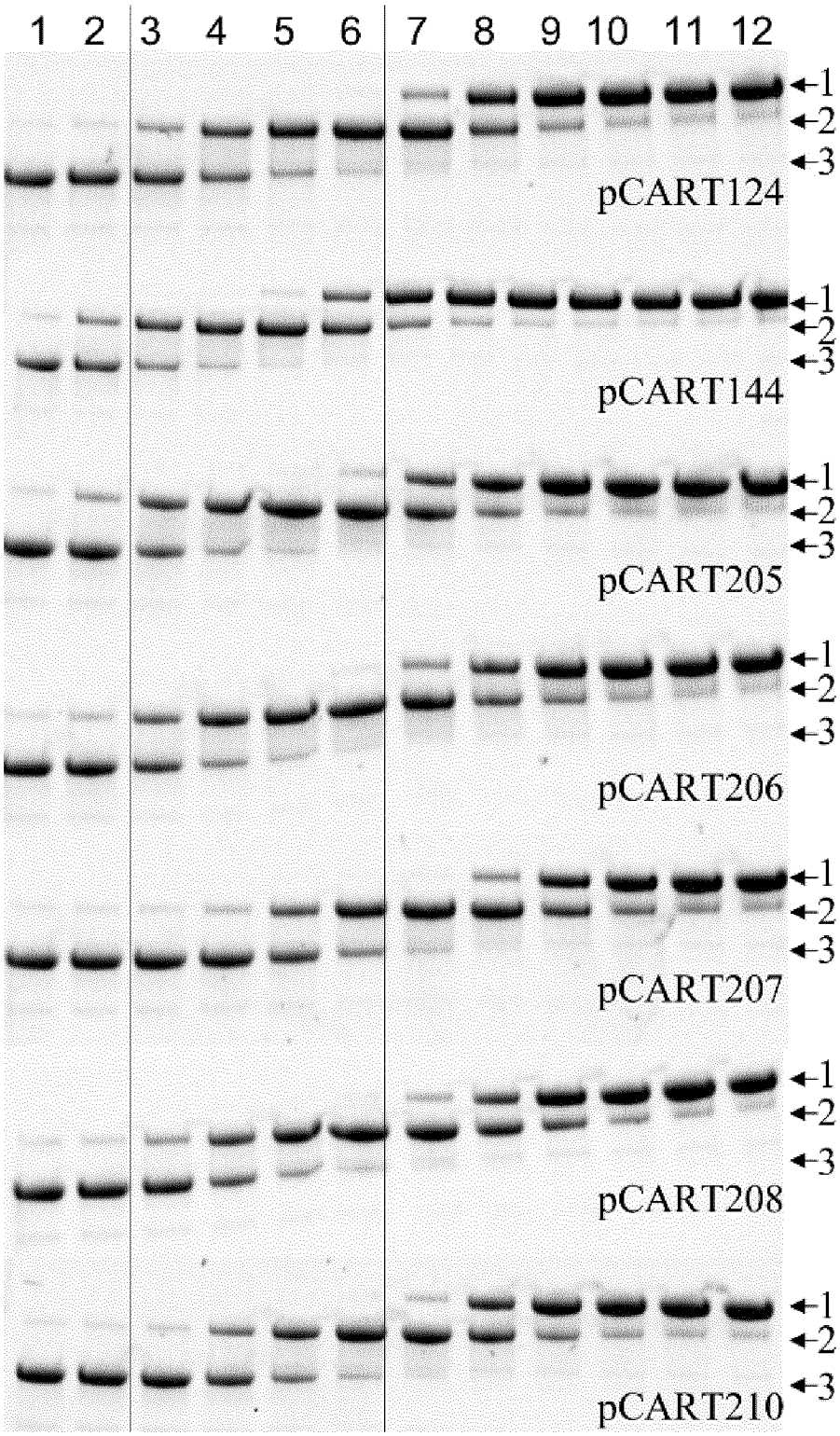
FIGURE 5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pCART210

FIGURE 6

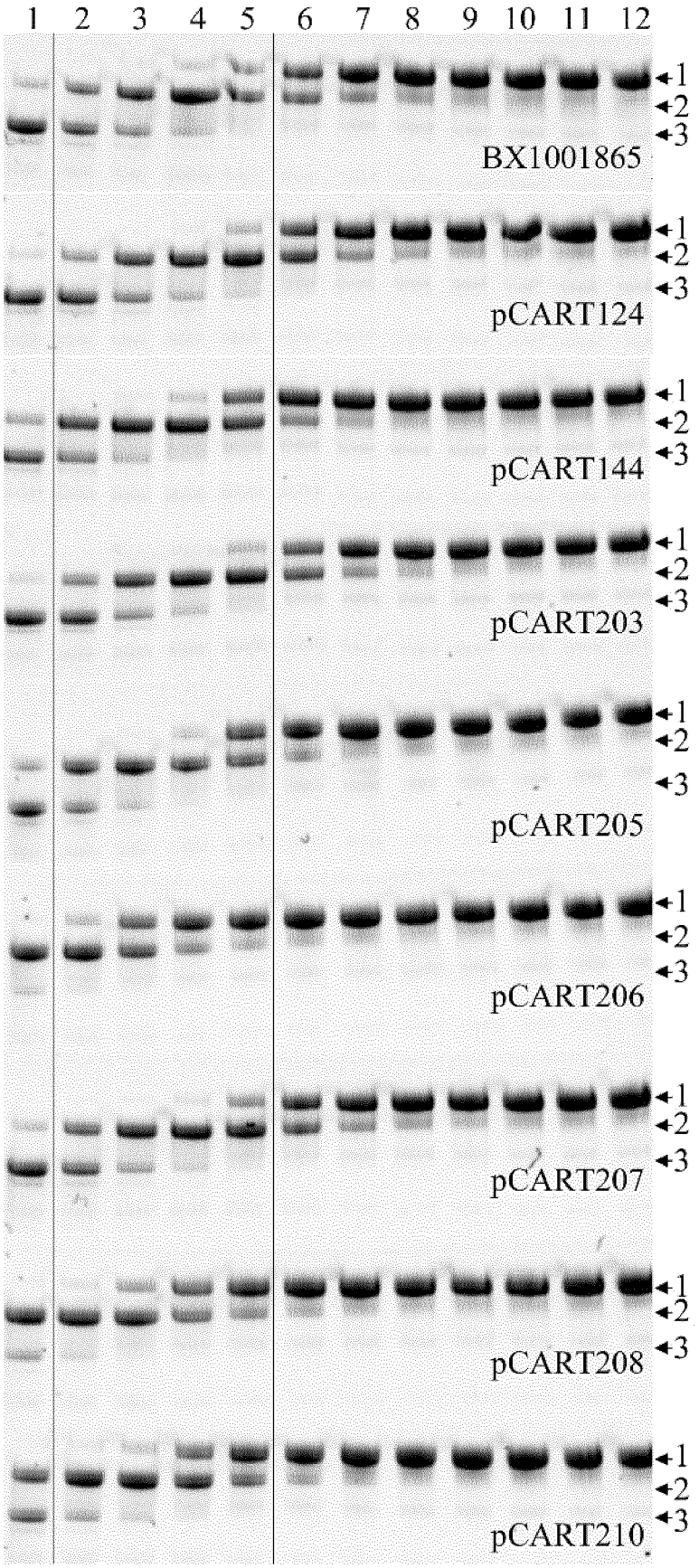
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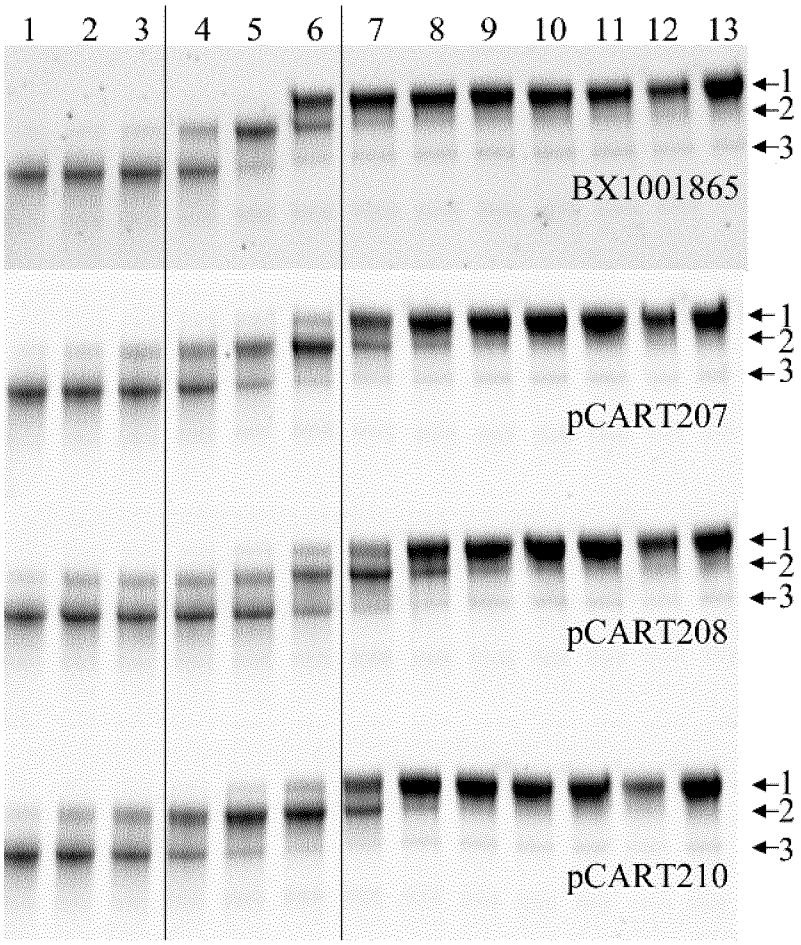
FIGURE 7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pCART210

FIGURE 8

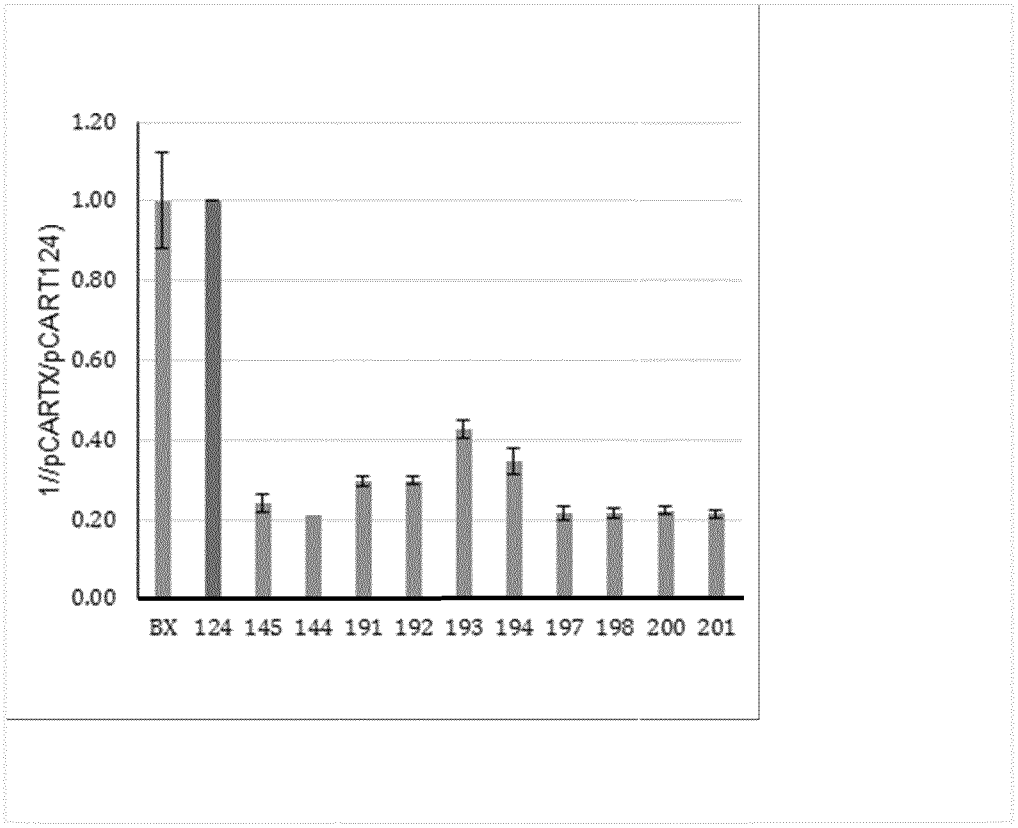


FIGURE 9

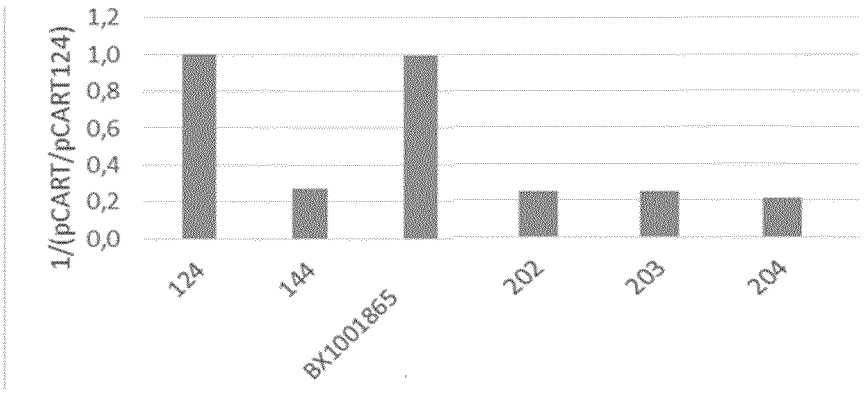


FIGURE 10

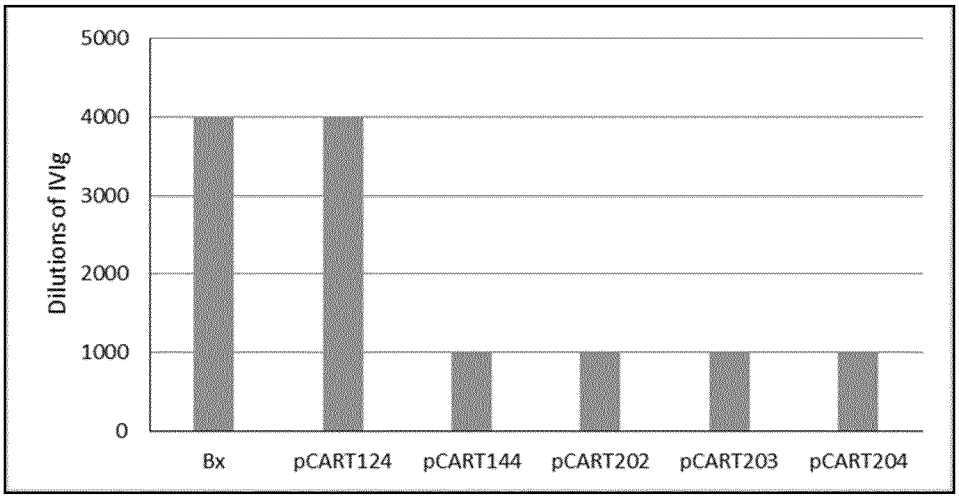


FIGURE 11

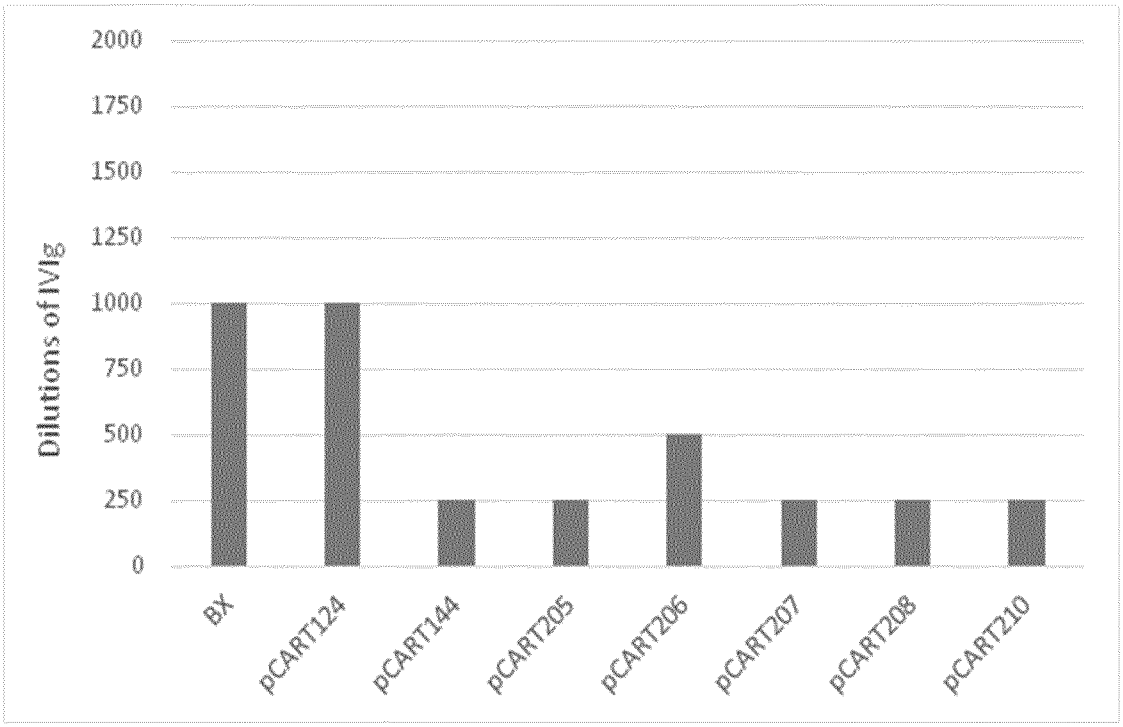


FIGURE 12

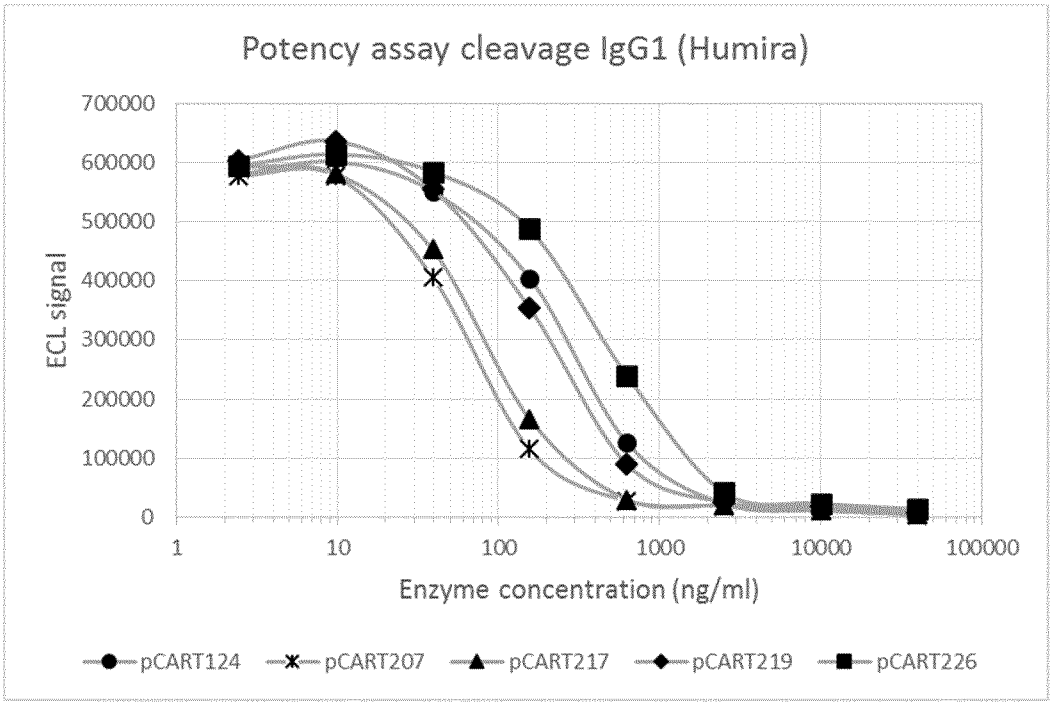


FIGURE 13

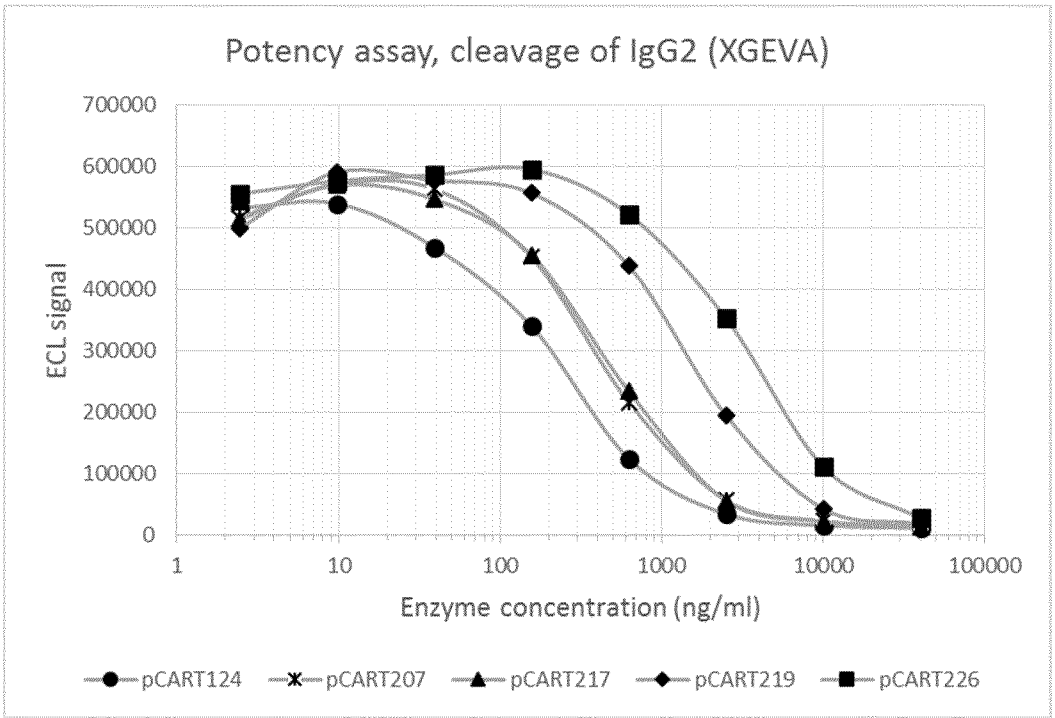


FIGURE 14

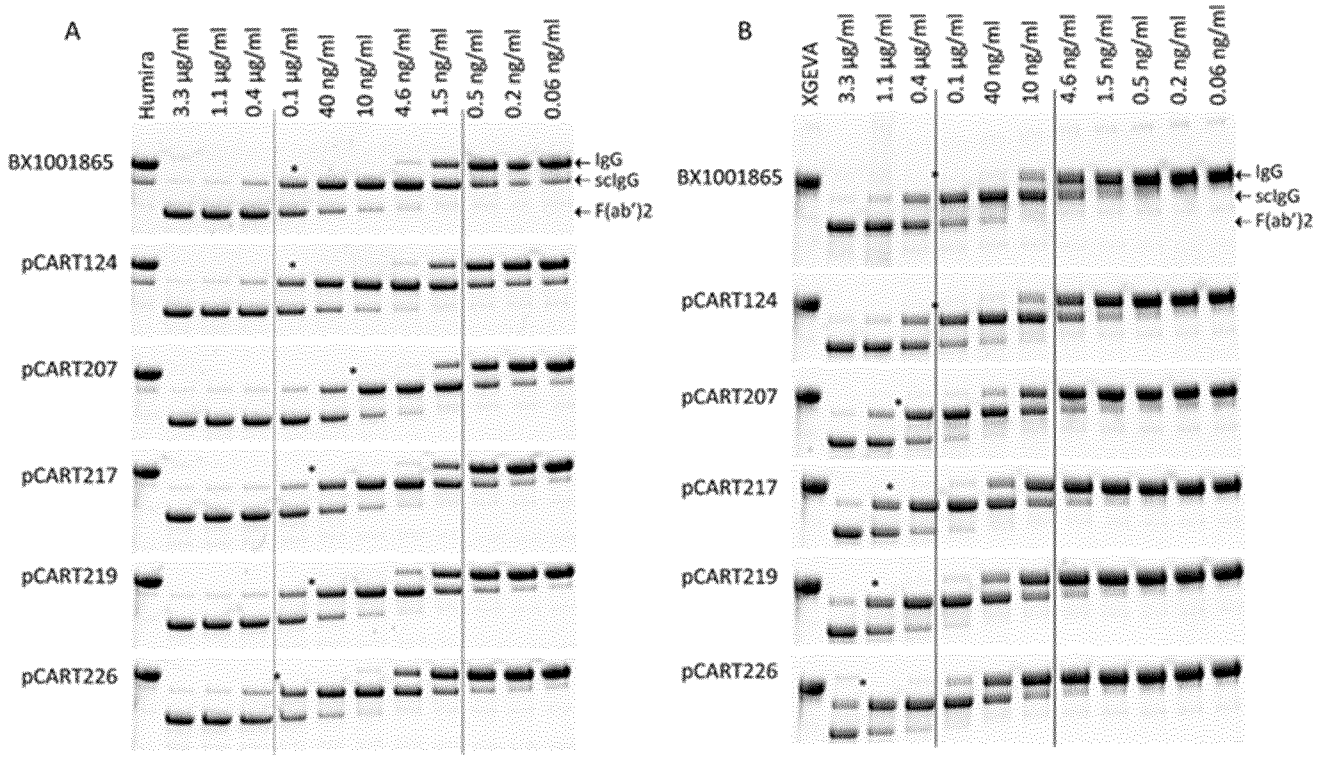


FIGURE 15

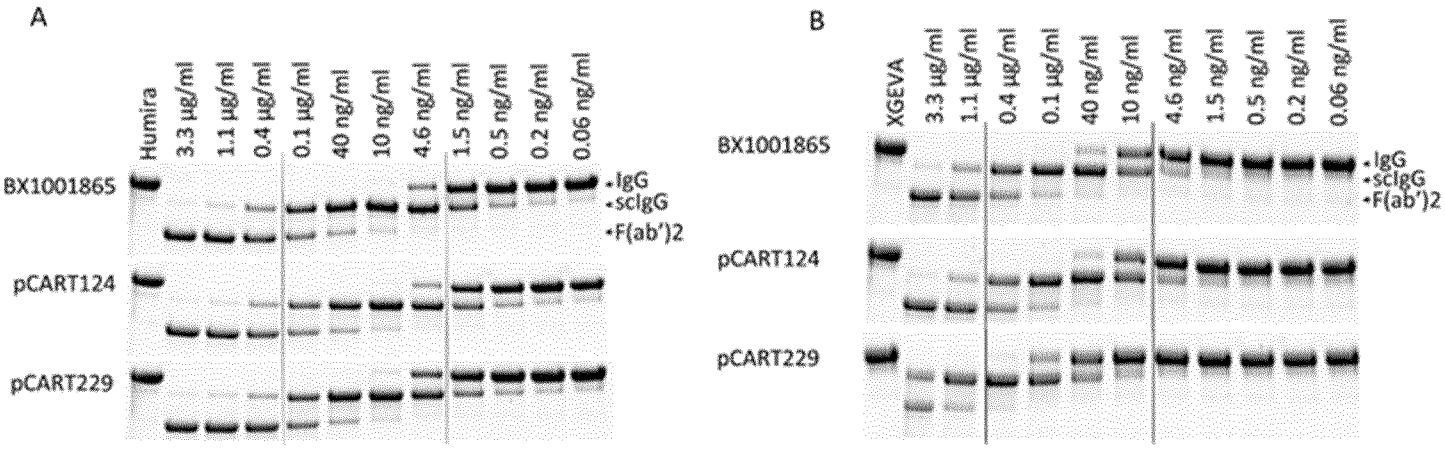


FIGURE 16

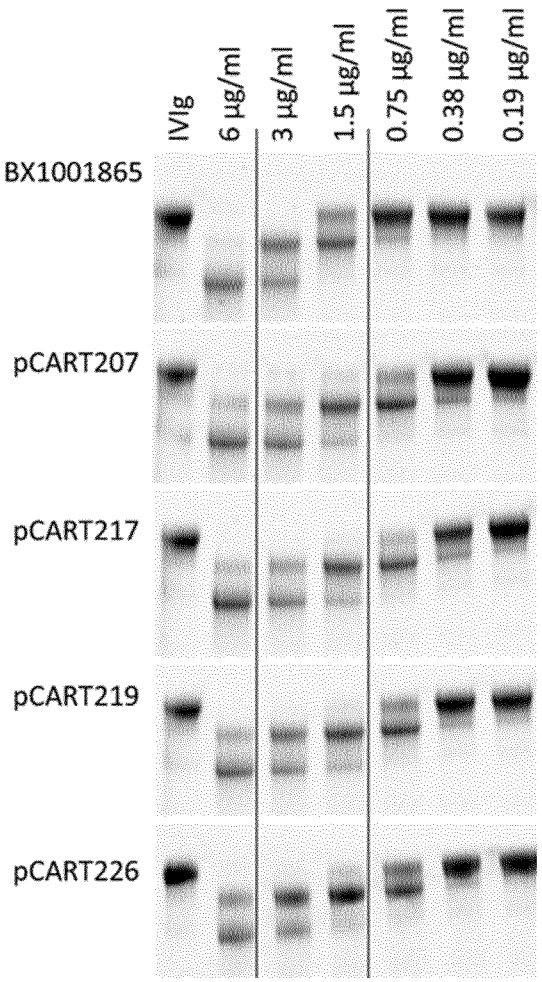


FIGURE 17

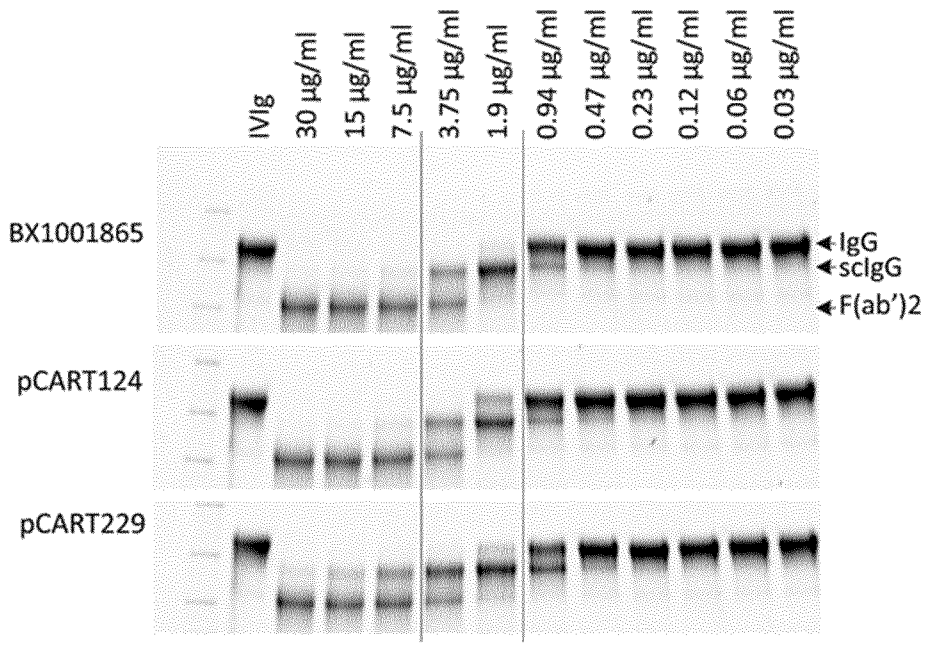


FIGURE 18

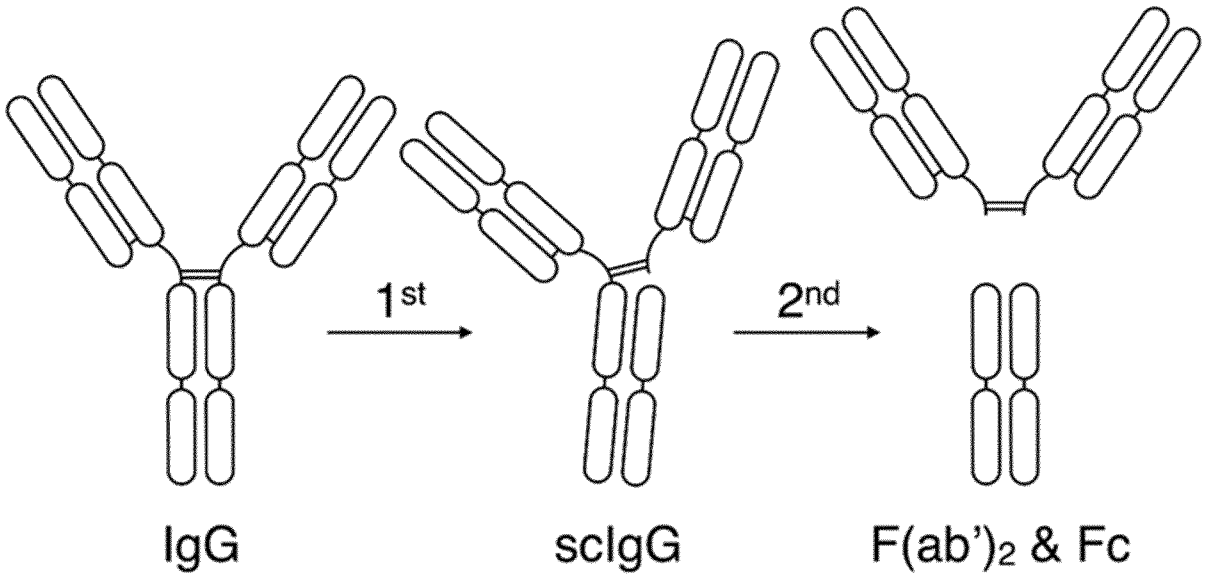


FIGURE 19

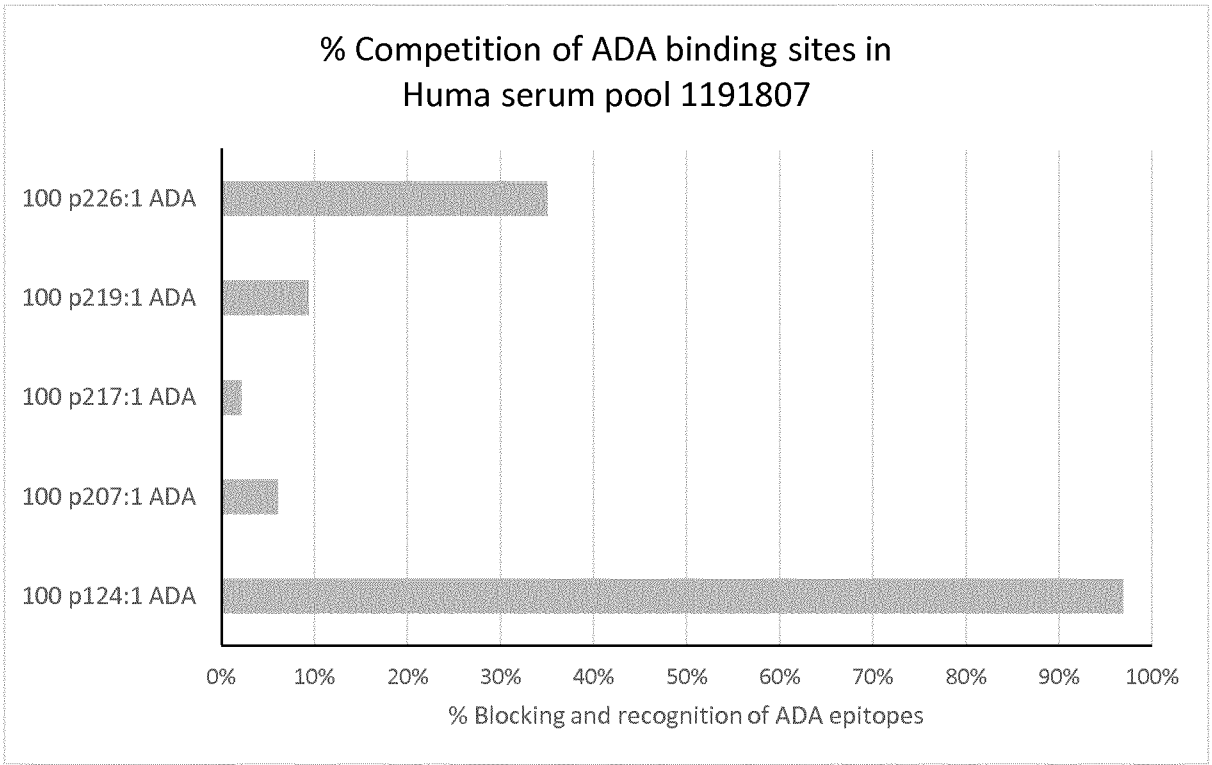


FIGURE 20

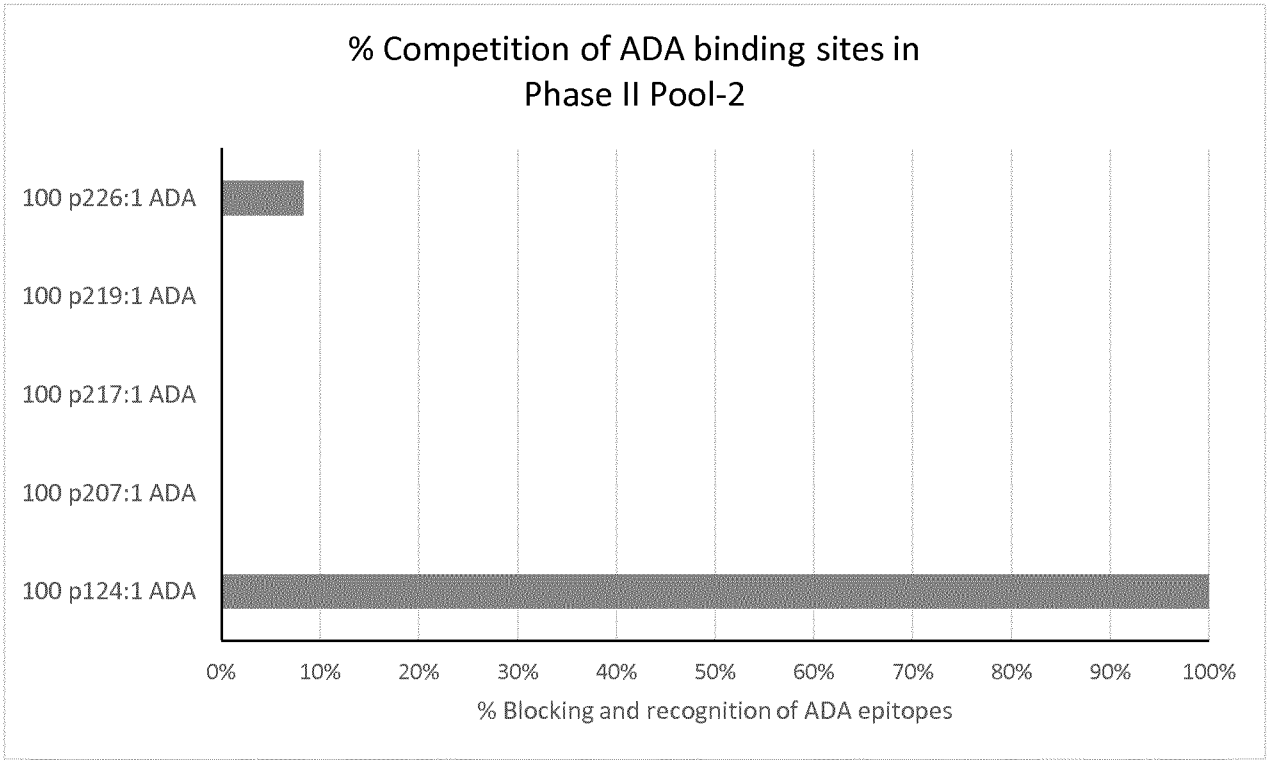


FIGURE 21

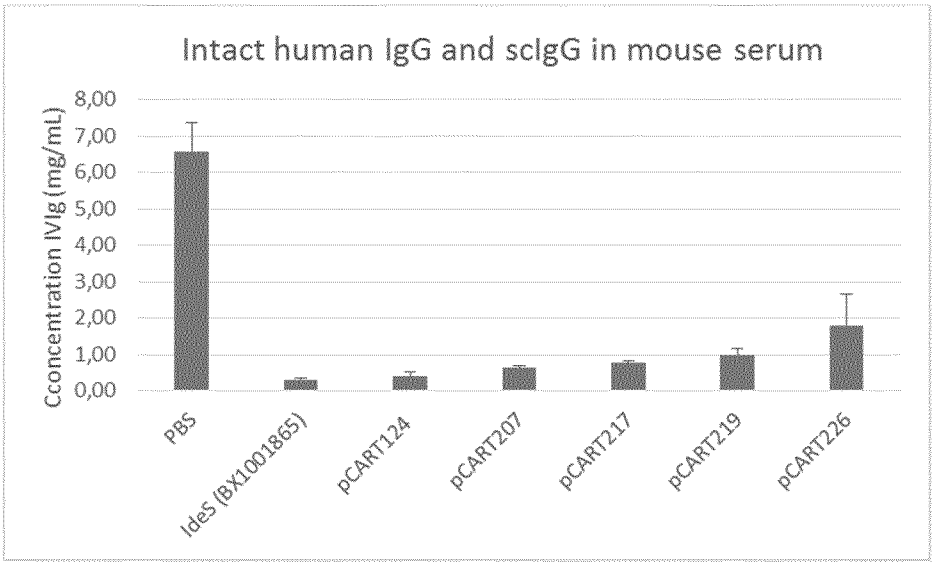
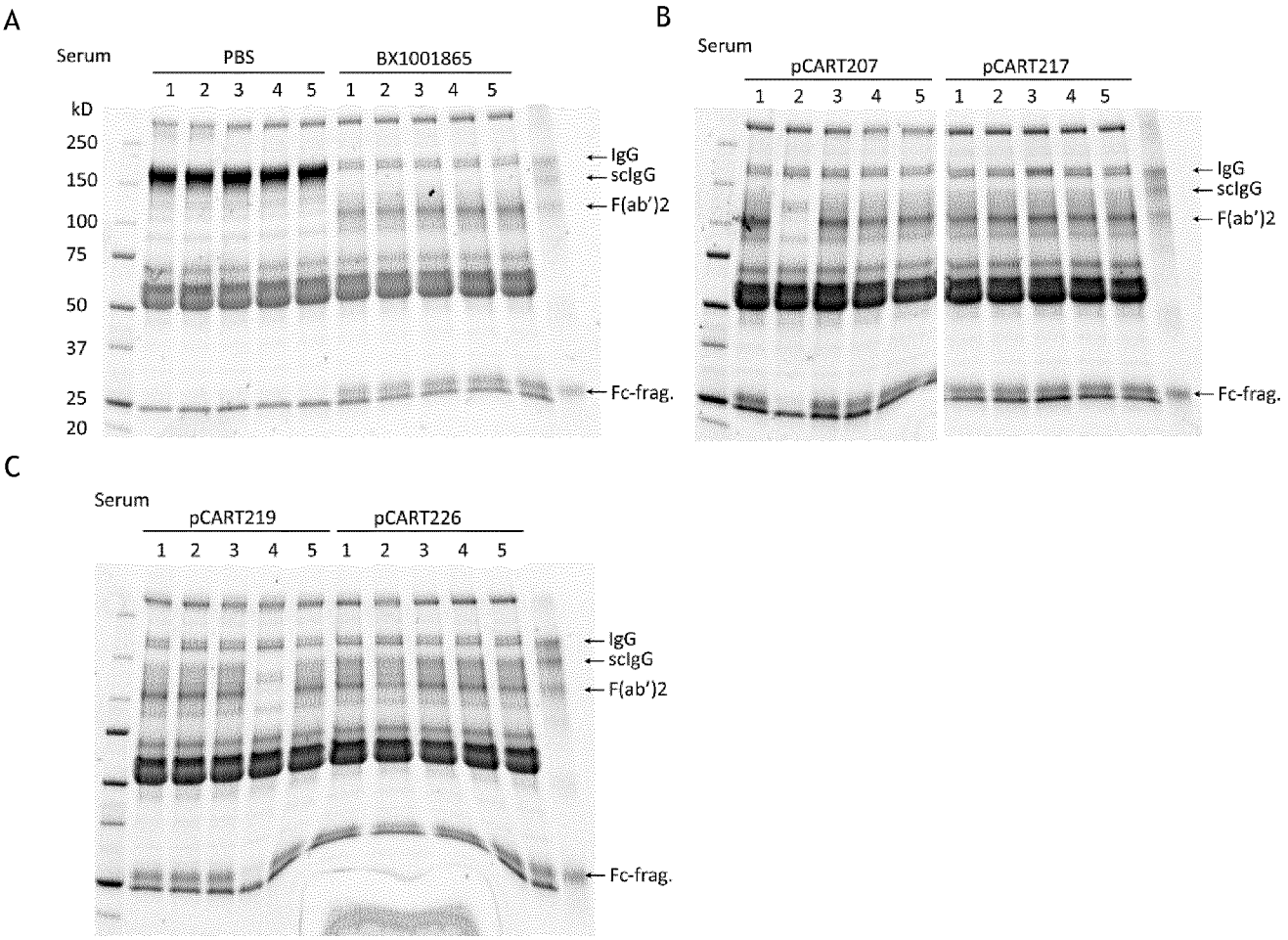
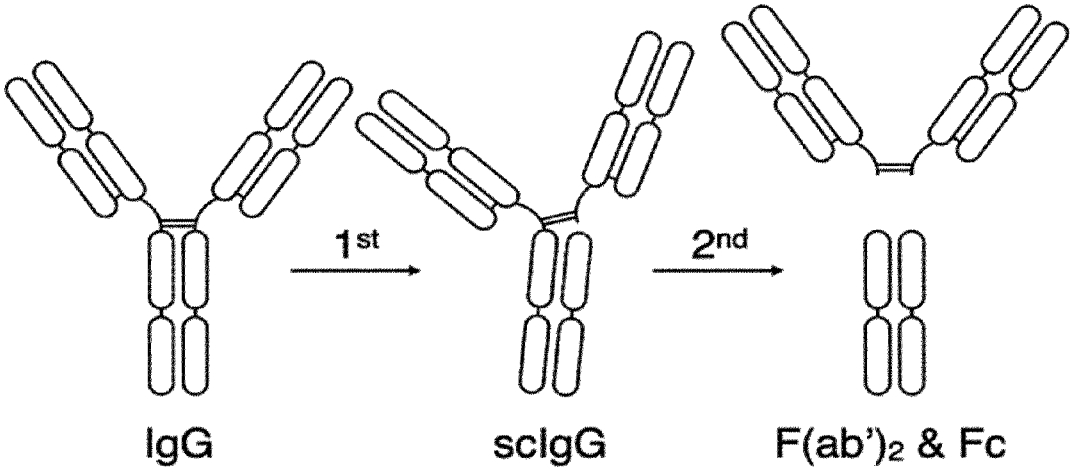


FIGURE 22


A
Serum
PBS
BX1001865
1 2 3 4 5 1 2 3 4 5
kD
250
150
100
75
50
37
25
20
IgG
scIgG
F(ab')2
Fc-frag.
B
Serum
pCART207
pCART217
1 2 3 4 5 1 2 3 4 5
IgG
scIgG
F(ab')2
Fc-frag.
C
Serum
pCART219
pCART226
1 2 3 4 5 1 2 3 4 5
IgG
scIgG
F(ab')2
Fc-frag.


1st
2nd
IgG
scIgG
F(ab')2 & Fc