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(54) Title: COMBINING CD27 AGONISTS AND IMMUNE CHECKPOINT INHIBITION FOR IMMUNE STIMULATION

(57) Abstract: The present invention relates to treatments of conditions ameliorated by stimulation of an immune response, in particular by the stimulation of antigen-specific T-lymphocytes. Treatment of such conditions according to the invention is effected by the combination of an anti-human CD27 agonistic antibody together with a number of immune checkpoint inhibitors.

Combining CD27 agonists and immune checkpoint inhibition for immune stimulation

FIELD OF THE INVENTION

The present invention relates to the field of human and veterinary medicine, including medical/veterinary diagnosis and medical/veterinary research. More specifically the present invention relates to treatments of conditions ameliorated by stimulation of an immune response, in particular by the stimulation of antigen-specific T-lymphocytes. The various aspects of the present invention are suitable for treatment of any condition known or expected to be ameliorated by stimulation of CD27+ immune cells or by inhibition of one or more immune checkpoint protein(s). Conditions suitably treated by the invention are those ameliorated by immune stimulation, such as infectious diseases and cancers, by using a combination of a CD27 agonist and one or more immune checkpoint blocking agents.

BACKGROUND

CD27, a TNF receptor family member was identified as a membrane molecule on human T cells (van Lier et al., 1987, J Immunol 139:1589-96). According to current evidence, CD27 has a single ligand, CD70, which is also a TNF family member (Goodwin et al., 1993, *Cell* 73:447-56).

CD27 is exclusively expressed by hematopoietic cells, in particular those of the lymphocyte lineage, i.e. T-, B- and NK cells. CD27 was originally defined as a human T-cell co-stimulatory molecule that increments the proliferative response to TCR stimulation (van Lier et al., 1987, *J Immunol* 139:1589-96). Presence of CD70, the ligand of CD27, dictates the timing and persistence of CD27-mediated co-stimulation.

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Transgenic expression of CD70 in immature dendritic cells sufficed to convert immunological tolerance to virus or tumors into CD8^{+} T cell responsiveness. Likewise, agonistic soluble CD70 promoted the CD8^{+} T cell response upon

5   such peptide immunization (Rowley et al., 2004, *J Immunol* 172:6039-6046) and in CD70 transgenic mice, CD4^{+} and CD8^{+} effector cell formation in response to TCR stimulation was greatly facilitated (Arens et al. 2001, *Immunity* 15:801-12; Tesselaar et al., 2003, *Nat Immunol* 4:49-54; Keller et al.

10   2008, *Immunity* 29: 334-346). In mouse lymphoma models, tumor rejection was improved upon CD70 transgenesis or injection of an anti-mouse CD27 antibody (Arens et al., 2003, *J Exp Med* 199:1595-1605; French et al., 2007, *Blood* 109: 4810-15; Sakanishi and Yagita, 2010, Biochem. Biophys. Res. Comm.

15   393: 829-835; WO 2008/051424; WO 2012/004367).

In WO2012/004367 the first anti-human agonistic antibody (designated hCD27.15) was described that does not require crosslinking to activate CD27-mediated co-stimulation of the immune response. In addition, an anti-

20   human CD27 antibody, designated 1F5 was disclosed that activates CD27 upon crosslinking (WO2011/130434 and Vitale et al., *Clin. Cancer Res*, 2012, 18(14): 3812-3821).

Recently, the first clinical successes of agents that modulate cancer immunity have validated cancer

25   immunotherapy as a novel path to obtain durable and long-lasting clinical responses in cancer patients (Mellman *et al., Nature*, 2011, 480:480-489). The first such agent, ipilimumab (Yervoy, BMS), that obtained marketing approval for treatment of metastatic melanoma is an antibody blocking

30   the CTLA4 receptor, an immune checkpoint protein. Further immune checkpoint inhibitors under development are antibodies that block the interaction between the PD-1 receptor and its ligands PD-L1 and PD-L2 (Mullard, Nat. Rev.

Drug Disc., 2013, 12:489-492). Several antibodies targeting
the PD-1 pathway are currently in clinical development for
treatment of melanoma, renal cell cancer, non-small cell
lung cancer, diffuse large B cell lymphoma and other tumors.

5   Although these agents have not yet been filed for marketing
approval, impressive results have been obtained in early
clinical studies, for example with Lambrolizumab (anti-PD1)
in melanoma (Hamid et al., 2013, New. Eng. J. Med., 369:134-
44).

10   The current state of the art does not suggest that
agonists of the CD27 receptor would be rationally combined
with immune checkpoint inhibitors, such as anti-PD1, anti-
PDL1 or anti-CTLA4 antibodies to improve cancer immunity. In
fact, available data suggest that CD27 acts, at least in

15   part via overruling PD-1 and CTLA-4 signals or via
downregulation of these immune checkpoints. First, in a T-
cell tolerance model based on LCMV-derived epitopes that was
demonstrated to be highly dependent on PD-1 and CTLA4
receptors the forced expression of CD70 ligand was

20   sufficient to turn T cell tolerance into activation of T-
cell immunity. Apparently these data strongly suggest that
CD27 activation overrules tolerance mediated via PD-1 and
CTLA4 (Keller et al., Immunity, 2008, 29:934-946. In a
second model, activation of CD27 using a rat anti-mouse CD27

25   agonistic antibody was demonstrated to support the
maintenance of CD8$^+$ T-cells, to reduce the frequency of
FoxP3-expressing CD4$^+$ T-cells within tumors and to potentiate
the ability of NK1.1$^+$ and CD8$^+$ tumor infiltrating cells to
secrete IFN-γ in coculture with tumor cells. This enhanced

30   function correlated with decreased levels of PD-1 expression
on CD8$^+$ T-cells (Roberts et al., J Immunother., 2010, 33:
769-79).

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SUMMARY

The current state of the art thus is, that stimulation of CD27 overrules PD-1 and CTLA-4 mediated immune tolerance and that activation using anti-CD27 antibodies results in down-regulation of these immune checkpoint receptors. Therefore, according to the state of the art the combination of an agent that activates the CD27 receptor together with one or more immune checkpoint inhibitors would result in either efficacy that is equal or similar to CD27 activation or immune checkpoint inhibition alone.

The inventors of the present invention however have surprisingly found that the combination of a CD27 agonistic antibody together with an immune checkpoint inhibitor does have additional effects on T-cell stimulation in comparison to a CD27 agonistic antibody or an immune checkpoint inhibitor alone. In particular, the additional effects of the combination of a CD27 agonistic antibody together with an immune checkpoint inhibitor has been tested in established assays that have been clinically validated to predict anti-cancer immune responses. In these assays immune checkpoint inhibitory antibodies were demonstrated to induce elevated levels of the T-cell cytokine IL-2 upon stimulation with Staphylococcus Enterotoxin B in either human peripheral blood mononuclear cells or whole human blood (Dulos *et al.*, *J. Immunother*, 2012, 35:169-78). The clinical validation and the predictive value of this assay was subsequently established in Phase I/II clinical studies (Patnaik *et al.*, ASCO, Chicago, 2012; Ribas *et al.*, PEGS Summit, Boston, 2013; Hamid et al., *N. Engl. J. Med.*, 2013, 369: 134-144).

The present invention is thus based on the surprising discovery that the combination of an anti-human CD27 agonistic antibody together with an immune checkpoint

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inhibitor results in immune stimulation to an unexpected
level. In view of the so far known relation between CD27 and
immune checkpoint inhibitors the higher level of immune
stimulation resulting from the combination is surprising. In
view of its surprising discovery the present invention
relates to treatments of conditions ameliorated by
stimulation or enhancement of the immune response, in
particular the treatments of conditions that result in the
stimulation or enhancement of antigen-specific T-
lymphocytes, such as cancer and infectious disease. More
specifically, the present invention is aimed at treatment of
any condition known or expected to be ameliorated by
stimulation of CD27+ immune cells or by inhibition of one or
more immune checkpoint protein(s). Treatment of these
conditions may be further improved by using a combination of
a CD27 agonist and one or more immune checkpoint blocking
agents.

     According to a first aspect the invention relates
to an anti-human CD27 agonistic antibody, such as hCD27.15
or 1F5, or an antibody derived therefrom, for use in the
treatment of a condition ameliorated by stimulation of an
immune response, in particular the treatment of a condition
ameliorated by stimulation of antigen-specific T-lymphocytes
wherein in said treatment a number of immune checkpoint
protein inhibitors is administered. By this co-
administration of the anti-human CD27 agonistic antibody
with a number of immune checkpoint protein inhibitors,
surprising effects are obtained.

     According to a further aspect the invention
relates to an immune checkpoint protein inhibitor for use in
the treatment of a condition ameliorated by stimulation of
an immune response, in particular the treatment of a
condition ameliorated by stimulation of antigen-specific T-

lymphocytes, wherein in said treatment an anti-human CD27 agonistic antibody, such as hCD27.15 or 1F5, or an antibody derived therefrom, is administered. By this co-administration of the immune checkpoint protein inhibitor

5   with an anti-human CD27 agonistic antibody, surprising effects are obtained.

Yet a further aspect of the invention relates to a combination of an anti-human CD27 agonistic antibody, such as hCD27.15 or 1F5, or an antibody derived therefrom,

10   together with a number of immune checkpoint protein inhibitors for use in the treatment of a condition ameliorated by stimulation of an immune response, in particular the treatment of a condition ameliorated by stimulation of antigen-specific T-lymphocytes.

15   Still a further aspect of the invention relates to a method for treating a condition ameliorated by stimulation of an immune response, in particular the treatment of a condition ameliorated by stimulation of antigen-specific T-lymphocytes, said method comprising administering an anti-

20   human CD27 agonistic antibody, such as hCD27.15 or 1F5, or an antibody derived therefrom, in combination with a number of immune checkpoint inhibitors.

An anti-human CD27 antibody of the invention may be selected from hCD27.15 or analogues thereof, in particular

25   analogues comprising the CDRs of hCD27.15, analogues (cross-)blocking the binding of hCD27.15 to human CD27, analogues binding to the same epitope of hCD27.15 or humanized analogues of hCD27.15.

In another embodiment, the anti-human CD27 antibody

30   is administered in combination with an anti-PD1 antibody. In one embodiment, the anti-human CD27 antibody is administered in combination with nivolumab. In another embodiment, the anti-human CD27 antibody is administered in

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combination with pembrolizumab. In another embodiment, the anti-human CD27 antibody is administered in combination with an anti-CTLA4 antibody. In another embodiment, the anti-human CD27 antibody is administered in combination with an anti-LAG3 antibody. In another embodiment, the anti-human CD27 antibody is administered in combination with an anti-LAG3 antibody comprising the heavy chain amino acid sequence of SEQ ID NO: 23 and the light chain amino acid sequence of SEQ ID NO: 24.

        As the skilled person will know the published sequences for the heavy and light chain of nivolumab and pembrolizumab are as presented in SEQ ID NO: 21, 22, 19 and 20 respectively.

Table 1: Sequence Listing

SEQ ID NO:	Description
1	hCD27.15 heavy chain CDR1 (AA)
2	hCD27.15 heavy chain CDR2 (AA)
3	hCD27.15 heavy chain CDR3 (AA)
4	hCD27.15 light chain CDR1 (AA)
5	hCD27.15 light chain CDR2 (AA)
6	hCD27.15 light chain CDR3 (AA)
7	hCD27.15 heavy chain variable region (DNA)
8	hCD27.15 heavy chain variable region (AA)
9	hCD27.15 light chain variable region (DNA)
10	hCD27.15 light chain variable region (AA)
11	1F5 heavy chain CDR1 (AA)
12	1F5 heavy chain CDR2 (AA)
13	1F5 heavy chain CDR3 (AA)
14	1F5 light chain CDR1 (AA)
15	1F5 light chain CDR2 (AA)
16	1F5 light chain CDR3 (AA)
17	1F5 heavy chain variable region (AA)

18	1F5 light chain variable region (AA)
19	Pembrolizumab heavy chain (AA)
20	Pembrolizumab light chain (AA)
21	Nivolumab heavy chain (AA)
22	Nivolumab light chain (AA)
23	Anti-human LAG3 mature heavy chain (AA)
24	Anti-human LAG3 mature light chain (AA)

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. CD27 agonistic antibodies in combination with immune checkpoint blockers unexpectedly enhance SEB induced T-cell activation in human PBMCs in comparison to CD27 agonistic antibodies and immune checkpoint inhibitors alone. Figure 1A. CD27 agonistic antibodies in combination with anti-PD1 antibodies enhance SEB induced T-cell activation in human PBMCs. Equal amounts of the indicated antibodies were added, with a final concentration as indicated on the X-axis. Human IgG4 was used as an isotype-matched control. Figure 1B. CD27 agonistic antibodies in combination with anti-PDL1 antibodies enhance SEB induced T-cell activation in human PBMCs. Equal amounts of the indicated antibodies were added, with a final concentration as indicated on the X-axis. Human IgG4 and/or mouse IgG1 were used as isotype-matched controls.

Figure 2. CD27 agonistic antibodies in combination with immune checkpoint blockers unexpectedly enhance SEB induced T-cell activation in human whole blood in comparison to CD27 agonistic antibodies and immune checkpoint inhibitors alone. Figure 2A. CD27 agonistic antibodies in combination with anti-PD1 antibodies enhance SEB induced T-cell activation in human whole blood. Equal amounts of the indicated antibodies were added, with a final concentration as indicated on the X-axis. Human IgG4 was used as isotype-matched control. Figure 2B. CD27 agonistic antibodies in

combination with anti-PDL1 antibodies enhance SEB induced T-cell activation in human whole blood. Equal amounts of the indicated antibodies were added, with a final concentration as indicated on the X-axis. Human IgG4 and/or mouse IgG1 were used as isotype-matched controls. Figure 2C. CD27 agonistic antibodies in combination with anti-CTLA-4 antibodies enhance SEB induced T-cell activation in human whole blood. Equal amounts of the indicated antibodies were added, with a final concentration as indicated on the X-axis. Human IgG4 and/or mouse IgG2A were used as isotype-matched controls.

Figure 3. CD27 agonistic antibodies in combination with immune checkpoint blockers enhance SEB induced T-cell activation in human whole blood in comparison to CD27 agonistic antibodies and immune checkpoint inhibitors alone. Figures 3A-3D present data for the effect of a hCD27.15 analogue in combination with anti-LAG3 (left panel) and anti-PD1 (right panel). Combination of the antibodies enhances SEB induced T-cell activation in human whole blood of different donors.

DETAILED DESCRIPTION

In a first aspect the invention relates to an anti-human CD27 agonistic antibody for use in the treatment of a condition ameliorated by stimulation of an immune response, in particular the treatment of a condition ameliorated by stimulation of antigen-specific T-lymphocytes, wherein in said treatment a number of immune checkpoint protein inhibitors is administered.

Anti-human CD27 agonistic antibodies should be construed as meaning an antibody demonstrating activation of the CD27 receptor on CD27$^+$ immune cells. Agonistic properties

of anti-human CD27 antibodies can be assayed by CD27
receptor activation using for example the NF-κB luciferase
reporter assay as described in WO2012/004367. Activation of
CD27 receptor using activating anti-human CD27 antibodies
has been shown to induce activation, proliferation and/or
survival of human CD27$^+$ immune cells. By displaying its CD27
receptor-stimulating effect the CD27 agonist is capable of
inducing and/or enhancing an immune response (e.g. an
antigen-specific T-cell mediated immune response). An anti-
human CD27 antibody used in the present invention may exert
its agonistic activity when in soluble form. Alternatively
an anti-human CD27 antibody used in the present invention
may exert its agonistic activity when cross-linked. For
cross-linking the anti-human CD27 antibody may be adapted in
respect of its Fc function. The use of anti-human CD27
antibodies exerting agonistic activity when in soluble form
is preferred.

Anti-human CD27 agonistic antibodies are known in
the art. For example hCD27.15 is disclosed in WO2012/004367
and 1F5 is disclosed in WO2011/130434 and Vitale et al.,
Clin. Cancer Res, 2012, 18(14): 3812-3821. The use of 1F5 or
hCD27.15 or an antibody derived from one of these known
antibodies is preferred. The use of hCD27.15 or an antibody
analogue therefrom is most preferred in view of its
beneficial binding properties and its ability to display
good agonistic activity when in soluble form (without any
further cross-linking). Within the present invention an
antibody derived from a certain antibody may be considered
an analogue. The skilled person will understand that for a
proper functioning of an antibody analogue within the
context of this invention, a derived antibody (or antibody
analogue), according to certain embodiments, will comprise
antigen binding regions of its originating antibody or will

at least bind to the same epitope. Antibody analogues may
(cross-)block binding of the anti-CD27 antibody, for example
hCD27.15 or 1F5, to human CD27. Antibody analogues in
particular comprise antibody fragments, antibodies having

5   modified effector function, chimeric antibodies and
humanized antibodies as defined below. The antibody analogue
according to the invention maintains CD27 agonistic
functionality.

          The heavy chain CDR1, CDR2 and CDR3 amino acid

10   sequences and light chain CDR1, CDR2 and CDR3 amino acid
sequences of hCD27.15, identifying the antigen binding
region of this antibody, are already disclosed in
WO2012/004367. These sequences have also been presented in
SEQ ID NO: 1-6 of the sequence listing of this description,

15   together with the amino acid sequences of the heavy chain
variable region and the light chain variable region (SEQ ID
NO: 8 and 10 respectively). Antibody analogues of hCD27.15
comprising these CDR sequences, or sequence variants
thereof, are particularly considered for use in the

20   invention.

          The heavy chain CDR1, CDR2 and CDR3 amino acid
sequences and light chain CDR1, CDR2 and CDR3 amino acid
sequences of 1F5, identifying the antigen binding region of
this antibody, are already disclosed in WO2011/130434. These

25   sequences have also been presented in SEQ ID NO: 11-16 of
the sequence listing of this description, together with the
amino acid sequences of the heavy chain variable region and
the light chain variable region (SEQ ID NO: 17 and 18).
Antibody analogues of 1F5 comprising these CDR sequences, or

30   sequence variants thereof, are particularly considered for
use in the invention.

          Alternatively, analogues of hCD27.15 or 1F5 binding
to the same epitope of these antibodies, but having

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differing CDRs may also be selected. To screen for antibodies that bind to the hCD27.15 or 1F5 epitope on human CD27, a routine cross-blocking assay such as that described in "Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988)", can be performed. Antibodies that bind to the same epitope are likely to cross-block in such assays, but not all cross-blocking antibodies will necessarily bind at precisely the same epitope since cross-blocking may result from steric hindrance of antibody binding by antibodies binding at overlapping epitopes, or even nearby non-overlapping epitopes. Such cross-blocking antibodies maintaining CD27 agonistic functionality are also within the scope of the present invention.

        Alternatively, the known technique of epitope mapping, e.g., as described in Champe *et al.*, 1995, *J. Biol. Chem.* 270:1388-1394, can be performed to determine whether the antibody binds an epitope of interest. "Alanine scanning mutagenesis," as described by Cunningham and Wells, 1989, *Science* 244: 1081-1085, or some other form of point mutagenesis of amino acid residues in human CD27 may also be used to determine the functional epitope for anti-CD27 antibodies of the present invention.

        Another known method to map the epitope of an antibody is to study binding of the antibody to synthetic linear and CLIPS peptides that can be screened using credit-card format mini PEPSCAN cards as described by Slootstra et al. (Slootstra et al., 1996, *Mol. Diversity* 1: 87-96) and Timmerman et al. (Timmerman et al., 2007, *J. Mol. Recognit.* 20: 283-299). The binding of antibodies to each peptide is determined in a PEPSCAN-based enzyme-linked immuno assay (ELISA).

Additional antibodies binding to the same epitope
as hCD27.15 may be obtained with known techniques, for
example, by screening of antibodies raised against CD27 for
binding to the epitope, or by immunization of an animal with
5   a peptide comprising a fragment of human CD27 comprising the
epitope sequences. Antibodies that bind to the same
functional epitope might be expected to exhibit similar
biological activities, such as CD27 agonistic activity, and
such activities can be confirmed by functional assays of the
10   antibodies. For analogues of 1F5 binding to the same epitope
of this antibody, these techniques can also be used in
analogy.

According to an embodiment an analogue of hCD27.15
binding to the same epitope of this antibody, but having
15   differing CDRs may block binding of hCD27.15 to human CD27
with an IC_{50} of about 50 nM or lower. Alternatively an
analogue of hCD27.15 binding to the same epitope of this
antibody, but having differing CDRs may be blocked in its
binding to human CD27 by hCD27.15 with an IC_{50} of about 50 nM
20   or lower. Similarly analogues of 1F5 binding to the same
epitope of this antibody, but having differing CDRs may
block binding of 1F5 to human CD27 with an IC_{50} of about 50
nM or lower or alternatively may be blocked in its binding
to human CD27 by 1F5 with an IC_{50} of about 50 nM or lower.
25   About 50 nM or lower is to be understood to include $50*10^{-9}$
to $0.1*10^{-12}$ M, such as $20*10^{-9}$ to $1.0*10^{-11}$ M, $10*10^{-9}$ to
$1.0*10^{-10}$ M or $10*10^{-9}$ to $1.0*10^{-19}$.

The differing CDRs may be sequence variants of the
known CDRs of the human CD27 binding antibody used in the
30   invention, for example hCD27.15 or 1F5. As used herein, a
sequence "variant" refers to a sequence that differs from
the disclosed sequence at one or more amino acid residues
but which retains the biological activity of the resulting

molecule. The invention includes the variants of antibodies explicitly disclosed by the various sequences, for example hCD27.15 or 1F5. For the V_H domain CDR1, CDR2 and CDR3 sequences, according to some embodiments, variant sequences may comprise up to 6 amino acid substitutions, such as 1, 2, 3, 4, 5 or 6 amino acid substitutions, for the CDR1, CDR2 and CDR3 sequences taken together. Similarly for the V_L domain CDR1, CDR2 and CDR3 sequences, according to some embodiments, variant sequences may comprise up to 6 amino acid substitutions, such as 1, 2, 3, 4, 5 or 6 amino acid substitutions, for the CDR1, CDR2 and CDR3 sequences taken together. The skilled person will understand that in particular conservative amino acid substitutions may result in maintaining biological activity. For all amino acid and DNA sequences disclosed, the sequence variants are also envisaged within this invention.

"Conservatively modified variants" or "conservative amino acid substitution" refers to substitutions of amino acids are known to those of skill in this art and may be made generally without altering the biological activity of the resulting molecule. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, e.g., Watson, et al., *Molecular Biology of the Gene*, The Benjamin/Cummings Pub. Co., p. 224 (4th Edition 1987)). Such exemplary substitutions are preferably made in accordance with those set forth above in **Table 2.**

Table 2: Exemplary Conservative Amino Acid Substitutions

Original residue	Conservative substitution
Ala (A)	Gly; Ser
Arg (R)	Lys, His

Asn (N)	Gln; His
Asp (D)	Glu; Asn
Cys (C)	Ser; Ala
Gln (Q)	Asn
Glu (E)	Asp; Gln
Gly (G)	Ala
His (H)	Asn; Gln
Ile (I)	Leu; Val
Leu (L)	Ile; Val
Lys (K)	Arg; His
Met (M)	Leu; Ile; Tyr
Phe (F)	Tyr; Met; Leu
Pro (P)	Ala
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr; Phe
Tyr (Y)	Trp; Phe
Val (V)	Ile; Leu

The anti-human CD27 agonistic antibody of the invention is intended for use in the treatment of a condition ameliorated by stimulation of an immune response,

5   in particular the treatment of conditions which are ameliorated by stimulation of antigen-specific T-lymphocytes. Characteristic of this treatment is that a number of immune checkpoint protein inhibitors is administered. Thus the CD27 agonistic antibody is co-

10   administered with a number of immune checkpoint protein inhibitors. Within the present invention the term "number of" should be understood as meaning at least one or alternatively one or more, such as, 1, 2, 3, 4, 5 or 6.

The term "immune checkpoint protein" is known in

15   the art. Within the known meaning of this term it will be

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clear to the skilled person that on the level of "immune checkpoint proteins" the immune system provides inhibitory signals to its components in order to balance immune reactions. Known immune checkpoint proteins comprise CTLA-4,

5   PD1 and its ligands PD-L1 and PD-L2 and in addition LAG-3, BTLA, B7H3, B7H4, TIM3, KIR. The pathways involving LAG3, BTLA, B7H3, B7H4, TIM3, and KIR are recognized in the art to constitute immune checkpoint pathways similar to the CTLA-4 and PD-1 dependent pathways (see e.g. Pardoll, 2012. *Nature*

10   *Rev Cancer* 12:252-264; Mellman et al., 2011. *Nature* 480:480-489).

       Within the present invention an immune checkpoint protein inhibitor is any compound inhibiting the function of an immune checkpoint protein. Inhibition includes reduction

15   of function and full blockade. In particular the immune checkpoint protein is a human immune checkpoint protein. Thus the immune checkpoint protein inhibitor preferably is an inhibitor of a human immune checkpoint protein. Immune checkpoint proteins are described in the art (see for

20   instance Pardoll, 2012. *Nature Rev. cancer* 12: 252-264). The designation immune checkpoint includes the experimental demonstration of stimulation of an antigen-receptor triggered T lymphocyte response by inhibition of the immune checkpoint protein *in vitro* or *in vivo*, e.g. mice deficient

25   in expression of the immune checkpoint protein demonstrate enhanced antigen-specific T lymphocyte responses or signs of autoimmunity (such as disclosed in Waterhouse et al., 1995. *Science* 270:985-988; Nishimura et al., 1999. *Immunity* 11:141-151). It may also include demonstration of inhibition

30   of antigen-receptor triggered CD4+ or CD8+ T cell responses due to deliberate stimulation of the immune checkpoint protein in vitro or in vivo (e.g. Zhu et al., 2005. Nature Immunol. 6:1245-1252).

Preferred immune checkpoint protein inhibitors are antibodies that specifically recognize immune checkpoint proteins. A number of CTLA-4, PD1, PDL-1, PD-L2, LAG-3, BTLA, B7H3, B7H4, TIM3 and KIR inhibitors are known and in analogy of these known immune checkpoint protein inhibitors, alternative immune checkpoint inhibitors may be developed in the (near) future. For example ipilimumab is a fully human CTLA-4 blocking antibody presently marketed under the name Yervoy (Bristol-Myers Squibb). A second CTLA-4 inhibitor is tremelimumab (referenced in Ribas et al., 2013, *J. Clin. Oncol.* 31:616-22). Examples of PD-1 inhibitors include without limitation humanized antibodies blocking human PD-1 such as lambrolizumab (e.g. disclosed as hPD109A and its humanized derivatives h409A11, h409A16 and h409A17 in WO2008/156712; Hamid et al., *N. Engl. J. Med.* 369: 134-144 2013,), or pidilizumab (disclosed in Rosenblatt et al., 2011. *J Immunother.* 34:409-18), as well as fully human antibodies such as nivolumab (previously known as MDX-1106 or BMS-936558, Topalian et al., 2012. *N. Eng. J. Med.* 366:2443-2454, disclosed in US8008449 B2). Other PD-1 inhibitors may include presentations of soluble PD-1 ligand including without limitation PD-L2 Fc fusion protein also known as B7-DC-Ig or AMP-244 (disclosed in Mkrtichyan M, et al. *J Immunol.* 189:2338-47 2012) and other PD-1 inhibitors presently under investigation and/or development for use in therapy. In addition, immune checkpoint inhibitors may include without limitation humanized or fully human antibodies blocking PD-L1 such as MEDI-4736 (disclosed in WO2011066389 A1), MPDL3280A (disclosed in US8217149 B2) and MIH1 (Affymetrix obtainable via eBioscience (16.5983.82)) and other PD-L1 inhibitors presently under investigation. According to this invention an immune checkpoint inhibitor is preferably selected from a CTLA-4, PD-1 or PD-L1

inhibitor, such as selected from the known CTLA-4, PD-1 or
PD-L1 inhibitors mentioned above (ipilimumab, tremelimumab,
labrolizumab, nivolumab, pidilizumab, AMP-244, MEDI-4736,
MPDL3280A, MIH1). Known inhibitors of these immune
5   checkpoint proteins may be used as such or analogues may be
used, in particular chimerized, humanized or human forms of
antibodies.

As the skilled person will know, alternative and/or
equivalent names may be in use for certain antibodies
10   mentioned above. Such alternative and/or equivalent names
are interchangeable in the context of the present invention.
For example it is known that lambrolizumab is also known
under the alternative and equivalent names MK-3475 and
pembrolizumab.

15   The selection of an immune checkpoint inhibitor
from PD1 and PD-L1 inhibitors, such as a known PD-1 or PD-L1
inhibitor mentioned above, is more preferred and most
preferably a selection is made from a PD-1 inhibitor, such
as a known PD1 inhibitor mentioned above. In preferred
20   embodiments, the PD1 inhibitor is nivolumab or pembrolizumab
or another antagonist antibody against human PD1.

The invention also includes the selection of other
immune checkpoint inhibitors that are known in the art to
stimulate immune responses. This includes inhibitors that
25   directly or indirectly stimulate or enhance antigen-specific
T-lymphocytes. These other immune checkpoint inhibitors
include, without limitation, agents targeting immune
checkpoint proteins and pathways involving PD-L2, LAG3,
BTLA, B7H4 and TIM3. For example, human PD-L2 inhibitors
30   known in the art include MIH18 (disclosed in (Pfistershammer
et al., 2006. Eur J Immunol. 36:1104-13). Another example,
LAG3 inhibitors known in the art include soluble LAG3
(IMP321, or LAG3-Ig disclosed in WO2009044273 A2, and in

Brignon et al. 2009. Clin. Cancer Res. 15:6225-6231) as well
as mouse or humanized antibodies blocking human LAG3 (for
instance IMP701 disclosed in and derived from WO2008132601
A1), or fully human antibodies blocking human LAG3 (such as
5   disclosed in EP 2320940 A2). Another example is provided by
the use of blocking agents towards BTLA, including without
limitation antibodies blocking human BTLA interaction with
its ligand (such as 4C7 disclosed in WO2011014438). Yet
another example is provided by the use of agents
10   neutralizing B7H4 including without limitation antibodies to
human B7H4 (disclosed in WO 2013025779 A1, and in WO
2013067492 A1) or soluble recombinant forms of B7H4 (such as
disclosed in US20120177645 A1 or Anti-human B7H4 clone H74:
eBiocience # 14-5948). Yet another example is provided by
15   agents neutralizing B7-H3, including without limitation
antibodies neutralizing human B7-H3 (e.g. MGA271 disclosed
as BRCA84D and derivatives in US 20120294796 A1). Yet
another example is provided by agents targeting TIM3,
including without limitation antibodies targeting human TIM3
20   (e.g. as disclosed in WO 2013006490 A2 or the anti-human
TIM3, blocking antibody F38-2E2 disclosed by Jones et al., J
Exp Med. 2008 Nov 24;205(12):2763-79). Known inhibitors of
immune checkpoint proteins may be used in their known form
or analogues may be used, in particular chimerized forms of
25   antibodies, most preferably humanized forms.

        The invention also includes the selection of more
than one immune checkpoint inhibitor selected from CTLA-4,
PD-1 or PDL1 inhibitors for combination with an anti-human
CD27 agonistic antibody within the various aspects of the
30   invention. For example concurrent therapy of ipilimumab
(anti-CTLA4) with Nivolumab (anti-PD1) has demonstrated
clinical activity that appears to be distinct from that
obtained in monotherapy (Wolchok *et al.*, 2013, *N. Eng. J.*

20

Med., 369:122-33). Also included are combinations of agents that have been shown to improve the efficacy of checkpoint inhibitors, such as Lirilumab (also known as anti-KIR, BMS-986015 or IPH2102, as disclosed in US8119775 B2 and Benson

5   et al., Blood 120:4324-4333 (2012)) in combination with ipilimumab (Rizvi et al., ASCO 2013, and clinicaltrials.gov NCT01750580) or in combination with nivolumab (Sanborn et al., ASCO 2013, and clinicaltrials.gov NCT01714739), agents targeting LAG3 combined with anti-PD-1 (Woo et al., 2012

10 Cancer Res. 72:917-27) or anti-PD-L1 (Butler NS et al., Nat Immunol. 2011 13:188-95), agents targeting ICOS in combination with anti-CTLA-4 (Fu et al., Cancer Res. 2011 71:5445-54, or agents targeting 4-1BB in combination with anti-CTLA-4 (Curran et al., PLoS One. 2011 6(4):e19499).

15 Combinations of anti-human CD27 agonistic antibodies and immune checkpoint inhibitors envisaged within the various embodiments of the present invention include those presented in Table 3. In this table the names of anti-human CD27 agonistic antibodies and immune checkpoint inhibitors refers

20 to both the known compound and analogues thereof. For antibodies, analogues include analogues having modified Fc-fuction, chimerized antibodies and humanized antibodies. For antibodies preferably human or humanized forms are selected. CD27 agonist refers to an anti-human CD27 agonistic

25 antibody.

Table 3

COMBINATIONS OF ANTI-HUMAN CD27 AGONISTS AND IMMUNE CHECKPOINT PROTEIN INHIBITORS																				
	CTLA-4 inhibitor	Ipilimumab	tremelimumab	PD-1 inhibitor	Lambrolizimab (pembrolizumab/MK-3475)	Nivolumab	pidilizumab	AMP244	PD-L1 inhibitor	MEDI-4736	MPDL3280A	MIH1	PD-L2 inhibitor	MIH18	KIR inhibitor e.g. lirilumab	LAG-3 inhibitor e.g. IMP321, IMP701	BTLA inhibitor, e.g. 4C7	B7H4 inhibitor, e.g. H74, soluble B7H4	B7H3 inhibitor, e.g. MA271	TIM3 inhibitor, e.g. F38-2E2
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In the table above presenting combinations of anti-human CD27 antibodies with Immune checkpoint inhibitors of the invention, numbers indicated (N) refer to the number of immune checkpoint inhibitors (N=1 or N=2) combined with an anti-human CD27 agonistic antibody. When a number is presented the combination comprises the immune checkpoint inhibitor(s) listed in the column at the position of the number.

Most of the immune checkpoint protein inhibitors presently known are antibodies. In view of the advances in antibody technology the use of an antibody as an immune checkpoint protein inhibitor is preferred in the present invention. However, the use of alternative immune checkpoint inhibitors based on other technologies is also envisaged in alternative embodiments.

As the skilled person will know other technologies are available for developing binding compounds interfering with the function of proteins such as immune checkpoint

proteins and thus acting as an inhibitor. For example as the
skilled person will understand, a library of binding
peptides engineered on non-immunoglobulin protein scaffolds
can be used to select binding peptides that inhibit immune

5   checkpoint proteins. Examples of such protein scaffolds
include, but at not restricted to Adnectins, Affibodies,
Anticalins and DARPins (Gebauer and Skerra, *Current opinion
Chem. Biol.*, 2009, 13:245-255 and Caravella and Lugovskoy,
Current opinion Chem. Biol., 2010, 14:520-528). Selection

10   methods for example include phage display to identify
protein scaffolds that express peptides binding to immune
check point proteins. In addition, combinatorial peptide
libraries comprising peptides potentially presenting immune
checkpoint protein inhibitory functions may be screened for

15   suitable immune checkpoint protein inhibitors. From such a
library For example, one-bead-one-compound combinatorial
libraries expressing a broad set of peptides on beads, where
one bead is binding one peptide, may be used. After
selection procedures, beads are recovered and the peptide is

20   identified (Lam *et al.*, *Methods*, 1996, 9:482-93; Xiao *et
al.*, *Comb. Chem. High Throughput Screen*, 2013 16:441-8). For
example using mass-spectrometry methods.

In the present invention the term "antibody" is
used in the broadest sense and specifically covers, but is

25   not limited to, monoclonal antibodies (including full length
monoclonal antibodies), polyclonal antibodies, and
multispecific antibodies (e.g., bispecific antibodies) and
binding fragments thereof.

"Antibody fragment" and "antibody binding

30   fragment" mean antigen-binding fragments of an antibody,
typically including at least a portion of the antigen
binding or variable regions (e.g. one or more CDRs) of the
parental antibody. An antibody fragment retains at least

some of the binding specificity of the parental antibody. Typically, an antibody fragment retains at least 10% of the parental binding activity when that activity is expressed on a molar basis. Preferably, an antibody fragment retains at least 20%, 50%, 70%, 80%, 90%, 95% or 100% or more of the parental antibody's binding affinity for the target. Therefore, as is clear for the skilled person, "antibody fragments" in many applications may substitute antibodies and the term "antibody" should be understood as including "antibody fragments" when such a substitution is suitable. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')2, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules, e.g., sc-Fv, unibodies or duobodies (technology from Genmab); nanobodies (technology from Ablynx); domain antibodies (technology from Domantis); and multispecific antibodies formed from antibody fragments. Engineered antibody variants are reviewed in Holliger and Hudson, 2005, Nat. Biotechnol. 23:1126-1136.

An "Fab fragment" is comprised of one light chain and the CH1 and variable regions of one heavy chain. The heavy chain of a Fab molecule cannot form a disulfide bond with another heavy chain molecule.

An "Fc" region contains two heavy chain fragments comprising the C_H1 and C_H2 domains of an antibody. The two heavy chain fragments are held together by two or more disulfide bonds and by hydrophobic interactions of the C_H3 domains.

An "Fab' fragment" contains one light chain and a portion of one heavy chain that contains the V_H domain and the C_H1 domain and also the region between the C_H1 and C_H2 domains, such that an interchain disulfide bond can be formed between the two heavy chains of two Fab' fragments to form a $F(ab')_2$ molecule.

28

An "F(ab')$_2$ fragment" contains two light chains and two heavy chains containing a portion of the constant region between the C_H1 and C_H2 domains, such that an interchain disulfide bond is formed between the two heavy chains. A

5   F(ab')$_2$ fragment thus is composed of two Fab' fragments that are held together by a disulfide bond between the two heavy chains.

The "Fv region" comprises the variable regions from both the heavy and light chains, but lacks the constant

10   regions.

A "single-chain Fv antibody" (or "scFv antibody") refers to antibody fragments comprising the V_H and V_L domains of an antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide

15   further comprises a polypeptide linker between the V_H and V_L domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv, see Pluckthun, 1994, The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds. Springer-Verlag, New York, pp. 269-

20   315. See also, International Patent Application Publication No. WO 88/01649 and U.S. Pat. Nos. 4,946, 778 and 5,260,203.

A "diabody" is a small antibody fragment with two antigen-binding sites. The fragments comprises a heavy chain variable domain (V_H) connected to a light chain variable

25   domain (V_L) in the same polypeptide chain (V_H-V_L or V_L-V_H). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described

30   more fully in, e.g., EP 404,097; WO 93/11161; and Holliger et al., 1993, Proc. Natl. Acad. Sci. USA 90: 6444-6448.

A "domain antibody fragment" is an immunologically functional immunoglobulin fragment containing only the

variable region of a heavy chain or the variable region of a
light chain. In some instances, two or more V_H regions are
covalently joined with a peptide linker to create a bivalent
domain antibody fragment. The two V_H regions of a bivalent

5   domain antibody fragment may target the same or different
antigens.

An antibody fragment of the invention may comprise
a sufficient portion of the constant region to permit
dimerization (or multimerization) of heavy chains that have

10 reduced disulfide linkage capability, for example where at
least one of the hinge cysteines normally involved in inter-
heavy chain disulfide linkage is altered with known methods
available to the skilled person. In another embodiment, an
antibody fragment, for example one that comprises the Fc

15 region, retains at least one of the biological functions
normally associated with the Fc region when present in an
intact antibody, such as FcRn binding, antibody half life
modulation, ADCC (antibody dependent cellular cytotoxicity)
function, and/or complement binding (for example, where the

20 antibody has a glycosylation profile necessary for ADCC
function or complement binding).

The antibody is directed against human CD27 and
thus comprises binding domains that bind to and/or interact
with human CD27. The antibody may be raised in an animal

25 from a non-human species suitable for eliciting antibodies
against human antigens. Alternatively, the antibody may be
isolated from antibody phage libraries generated using the
techniques described in McCafferty *et al.*, 1990, *Nature*,
348:552-554. Clackson *et al.*, 1991, *Nature*, 352:624-628, and

30 Marks *et al.*, 1991, *J. Mol. Biol.* 222:581-597. The skilled
person will be able to select a suitable non-human species
for eliciting antibodies against human antigens. For example
a selection may be made from a non-human mammal, such as a

rodent, including murine (rat or mouse) or hamster species, or alternatively a camelid species.

The antibody, when raised in a non-human species, preferably is chimerized with methods and techniques known in the art to form a "chimeric antibody". The term "chimeric" antibody refers to antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (See, for example, U.S. Pat. No. 4,816,567 and Morrison et al., 1984, Proc. Natl. Acad. Sci. USA 81:6851-6855). Within the present invention a "chimeric antibody" preferably is a "humanized antibody".

As used herein, the term "humanized antibody" refers to forms of antibodies that contain sequences from non-human (e.g., murine) antibodies as well as human antibodies. Such antibodies contain minimal sequence derived from non-human immunoglobulin. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. The humanized forms of rodent antibodies will essentially comprise the same CDR sequences of the parental rodent

antibodies, although certain amino acid substitutions may be included to increase affinity, increase stability of the humanized antibody, or for other reasons However, as CDR loop exchanges do not uniformly result in an antibody with

5   the same binding properties as the antibody of origin, changes in framework residues (FR), residues involved in CDR loop support, might also be introduced in humanized antibodies to preserve antigen binding affinity (Kabat et al., 1991, J. Immunol. 147:1709).

10   The term "antibody" also includes "fully human" antibodies, i.e., antibodies that comprise human immunoglobulin protein sequences only. A fully human antibody may contain non-human, such as murine (rat or mouse) carbohydrate chains if produced in a non-human cell

15   (e.g. mouse or hamster), or in a hybridoma derived from a murine cell. Similarly, "mouse antibody" or "rat antibody" refer to an antibody that comprises only mouse or rat immunoglobulin sequences, respectively.

               A fully human antibody may be generated in a human

20   being, in a transgenic non-human animal having human immunoglobulin germline sequences, by phage display or other molecular biological methods. Also, recombinant immunoglobulins may also be made in transgenic mice. See Mendez et al., 1997, Nature Genetics 15:146-156. See also

25   Abgenix, Medarex, MeMo and Kymab technologies.

               The antibodies of the present invention also include antibodies with modified (or blocked) Fc regions to provide altered effector functions. See, e.g. U.S. Pat. No. 5,624,821; WO2003/086310; WO2005/120571; WO2006/0057702;

30   Presta, 2006, *Adv. Drug Delivery Rev.* 58:640-656; Vincent and Zurini, *Biotechnol. J.*, 2012, 7:1444-50; Kaneko and Niwa, *Biodrugs*, 2011, 25: 1-11. Such modification can be used to enhance or suppress various reactions of the immune

system, with possible beneficial effects in diagnosis and therapy. Alterations of the Fc region include amino acid changes (substitutions, deletions and insertions), glycosylation or deglycosylation, and adding multiple Fc.

5   Preferably, Fc regions displaying reduced Fc effector functions are used. The antibodies of the present invention also include antibodies that have a human IgG4 containing Fc regions. And/or Fc regions carrying a N297Q glycosylation deficient mutant are used.

10   Changes to the Fc can also alter the half-life of antibodies in therapeutic antibodies, and a longer half-life would result in less frequent dosing, with the concomitant increased convenience and decreased use of material. See Presta, 2005, J. Allergy Clin. Immunol.116:731 at 734-35.

15   The antibodies of the present invention, although less preferred, also include antibodies with intact Fc regions that provide full effector functions, e.g. antibodies of isotype IgG1, which induce complement-dependent cytotoxicity (CDC) or antibody dependent cellular

20   cytotoxicity (ADCC) in the a cell associated with the target for the antibody.

        The antibodies may also be conjugated (e.g., covalently linked) to molecules that improve stability of the antibody during storage or increase the half-life of the

25   antibody *in vivo*. Examples of molecules that increase the half-life are albumin (e.g., human serum albumin) and polyethylene glycol (PEG). Albumin-linked and PEGylated derivatives of antibodies can be prepared using techniques well known in the art. See, e.g. Chapman, 2002, Adv. Drug

30   Deliv. Rev. 54:531-545; Anderson and Tomasi, 1988, J. Immunol. Methods 109:37-42; Suzuki et al., 1984, Biochim. Biophys. Acta 788:248-255; and Brekke and Sandlie, 2003, Nature Rev. 2:52-62.

As used herein, the term "about" refers to a value
that is within an acceptable error range for the particular
value as determined by one of ordinary skill in the art,
which will depend in part on how the value is measured or
5   determined, i.e. the limitations of the measurement system.
For example, "about" can mean within 1 or more than 1
standard deviation per the practice in the art.
Alternatively, "about" or "comprising essentially of" can
mean a range of up to 20%. Furthermore, particularly with
10   respect to biological systems or processes, the terms can
mean up to an order of magnitude or up to 5-fold of a value.
When particular values are provided in the application and
claims, unless otherwise stated, the meaning of "about" or
"comprising essentially of" should be assumed to be within
15   an acceptable error range for that particular value.

The antibody can be selected from any class of
immunoglobulins, including IgM, IgG, IgD, IgA, and IgE.
Preferably, the antibody is an IgG antibody. Any isotype of
IgG can be used, including IgG1, IgG2, IgG3, and IgG4.
20   Variants of the IgG isotypes are also contemplated. The
humanized antibody may comprise sequences from more than one
class or isotype. Optimization of the necessary constant
domain sequences to generate the desired biologic activity
is readily achieved by screening the antibodies in the
25   biological assays described in the Examples.

Likewise, either class of light chain can be used
in the compositions and methods herein. Specifically, kappa,
lambda, or variants thereof are useful in the present
compositions and methods.

30   The term "monoclonal antibody" as used herein
refers to an antibody obtained from a population of
substantially homogeneous antibodies, i.e., the individual
antibodies comprising the population are identical except

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for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal)

5   antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained

10   from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first

15   described by Kohler et al., 1975, *Nature* 256:495, or may be made by recombinant DNA methods (see, for example, U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson et al., 1991, *Nature* 352:624-628 and

20   Marks et al., 1991, *J. Mol. Biol.* 222:581-597, for example. The monoclonal antibodies herein specifically include "chimeric" antibodies.

Monoclonal antibodies can be made according to knowledge and skill in the art of injecting test subjects

25   with human antigen and then generating hybridomas expressing antibodies having the desired sequence or functional characteristics. DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of

30   binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a preferred source of such DNA.

35

For therapeutic applications the antibodies may be used as such or as a treatment conjugate. As used herein, a treatment "conjugate" refers to an antibody, or a fragment thereof, conjugated to a therapeutic moiety, such as a

5   bacterial toxin, a cytotoxic drug or a radiotoxin. Toxic moieties can be conjugated to antibodies, of the invention using methods available in the art.

In view of the fact that the present invention resides in the surprising immune stimulatory effects of the

10 combination of an anti-human CD27 agonistic antibody together with an immune checkpoint inhibitor, the present invention, in its various embodiments is suitable for treatment of a condition known or expected to be ameliorated by immune stimulation, in particular stimulation of antigen-

15 specific T-lymphocytes. In this invention the term antigen-specific T-lymphocyte in particular includes CD4+ and/or CD8+ T cells.

The immune stimulation, in particular the stimulation of antigen-specific T-lymphocytes, may be

20 achieved by stimulation of CD27$^+$ immune cells or by inhibition of an immune checkpoint protein, such as CTLA-4, PD1, PD-L1, PD-L2, LAG-3, BTLA, B7H3, B7H4, TIM3 and KIR. Thus according to certain embodiments the present invention, is suitable for treatment of a condition known or expected

25 to be ameliorated by stimulation of CD27$^+$ immune cells. According to certain alternative embodiments the present invention, is suitable for treatment of a condition known or expected to be ameliorated by inhibition of an immune checkpoint protein, such as CTLA-4, PD1, PD-L1, PD-L2, LAG-

30 3, BTLA, B7H3, B7H4, TIM3 or KIR.

The meaning of the term stimulation in the context of an immune response will be known to the skilled person. It will be clear that this term includes enhancement and

thus relates to both elevation of existing immune responses and induction or *de novo* generation of an immune response.

The skilled person will know which cells are associated with CD27, CTLA-4, PD1, PD-L1, PD-L2, LAG-3,

5   BTLA, B7H3, B7H4, TIM3 and KIR. In particular it is known that CD27 positive cells may or may not express on their cell surface CTLA-4, PD1, PD-L1, PD-L2, LAG-3, BTLA, B7H3, B7H4, TIM3 and KIR (and *vice versa*). Thus conditions treatable within the present invention are most certainly

10 not restricted to conditions involving cells expressing both CD27 and an immune checkpoint protein, such as CTLA-4, PD-1 or PD-L1, PD-L2, LAG-3, BTLA, B7H3, B7H4, TIM3 or KIR.

The various aspects of the invention are aimed at "treatment of a condition amelioratated by stimulation of an

15 immune response, in particular stimulation of antigen-specific T-lymphocytes". "The treatment of a condition amelioratated by stimulation of an immune response, in particular stimulation of antigen-specific T-lymphocytes" may alternatively be defined as "a treatment wherein

20 stimulation of an immune response, in particular stimulation of antigen-specific T-lymphocytes, is beneficial". The terms "amelioration" and "beneficial" in the context of treatment both refer to clinically meaning full improvement as can be predicted and/or established by a physician.

25 Within the present invention the treatment of the "condition" includes any therapeutic use including prophylactic and curative uses of the anti-human CD27 agonistic antibody and the number of immune checkpoint inhibitors. Therefore the term "condition" may refer to

30 disease states but also to physiological states in the prophylactic setting where physiology is not altered to a detrimental state. Conditions in the setting of prophylactic use of the anti-human CD27 agonistic antibody and the number

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of immune checkpoint inhibitors include for example immunization (the physiological process of inducing and/or generating immune memory against an antigen) after vaccination. The treatment within the context of the present

5   invention thus may be aimed at supporting prophylactically induced physiological processes.

Conditions ameliorated by immune stimulation, in particular stimulation of antigen-specific T-lymphocytes include infectious diseases, such as bacterial, fungal,

10   viral and parasitic infectious diseases. In addition immunization after vaccination may also be ameliorated by immune stimulation, in particular stimulation of antigen-specific T-lymphocytes. The vaccination may be against a pathogen, such as pathogen selected from bacteria, fungi,

15   viruses or parasites, or against toxins, or self-antigens, including antigens expressed on benign or malignant tumors, including cancers. Also conditions associated with uncontrolled proliferation of cells such as cancers may be ameliorated by immune stimulation, in particular stimulation

20   of antigen-specific T-lymphocytes. These conditions may be ameliorated by stimulation of CD27$^+$ immune cells and/or by inhibition of an immune checkpoint protein, such as CTLA-4, PD1, PD-L1, PD-L2, LAG-3, BTLA, B7H3, B7H4, TIM3 or KIR.

Cancers within the present invention include, but

25   are not limited to, leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, myeloblasts promyelocyte, myelomonocytic monocytic erythroleukemia, chronic leukemia, chronic myelocytic (granulocytic) leukemia, chronic lymphocytic leukemia, mantle cell lymphoma, primary central

30   nervous system lymphoma, Burkitt's lymphoma and marginal zone B cell lymphoma, Polycythemia vera Lymphoma, Hodgkin's disease, non-Hodgkin's disease, multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, solid

tumors, sarcomas, and carcinomas, fibrosarcoma, myxosarcoma, liposarcoma, chrondrosarcoma, osteogenic sarcoma, osteosarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon sarcoma, colorectal carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, non-small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, retinoblastoma, nasopharyngeal carcinoma, esophageal carcinoma, basal cell carcinoma, biliary tract cancer, bladder cancer, bone cancer, brain and central nervous system (CNS) cancer, cervical cancer, choriocarcinoma, colorectal cancers, connective tissue cancer, cancer of the digestive system, endometrial cancer, esophageal cancer, eye cancer, head and neck cancer, gastric cancer, intraepithelial neoplasm, kidney cancer, larynx cancer, liver cancer, lung cancer (small cell, large cell), melanoma, neuroblastoma; oral cavity cancer(for example lip, tongue, mouth and pharynx), ovarian cancer, pancreatic cancer, retinoblastoma, rhabdomyosarcoma, rectal cancer; cancer of the respiratory system, sarcoma, skin cancer,

stomach cancer, testicular cancer, thyroid cancer, uterine cancer, and cancer of the urinary system.

Less preferred cancers include CD27-expressing tumors, such as those selected from the group consisting of chronic lymphocytic leukemia, mantle cell lymphoma, primary central nervous system lymphoma, Burkitt's lymphoma and marginal zone B cell lymphoma.

Some examples of pathogenic viruses causing infections ameliorated by immune stimulation, in particular stimulation of antigen-specific T-lymphocytes include HIV, hepatitis (A, B, C, D or E), herpes virus (e.g., VZV, HSV-1, HAV-6, HSV-II, and CMV, Epstein Barr virus), adenovirus, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, cornovirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus and arboviral encephalitis virus.

Some examples of pathogenic bacteria causing infections ameliorated by immune stimulation, in particular stimulation of antigen-specific T-lymphocytes, include chlamydia, rickettsial bacteria, mycobacteria, staphylococci, streptococci, pneumonococci, meningococci and conococci, klebsiella, proteus, serratia, pseudomonas, legionella, diphtheria, salmonella, bacilli, cholera, tetanus, botulism, anthrax, plague, leptospirosis, and Lyme disease bacteria.

Some examples of pathogenic fungi causing infections ameliorated by immune stimulation, in particular stimulation of antigen-specific T-lymphocytes, include Candida (albicans, krusei, glabrata, tropicalis, etc.), Cryptococcus neoformans, Aspergillus (fumigatus, niger, etc.), Genus Mucorales (mucor, absidia, rhizophus),

Sporothrix schenkii, Blastomyces dermatitidis,
Paracoccidioides brasiliensis, Coccidioides immitis and
Histoplasma capsulatum.

Some examples of pathogenic parasites causing
5   infections ameliorated by immune stimulation, in particular
stimulation of antigen-specific T-lymphocytes, include
Entamoeba histolytica, Balantidium coli, Naegleriafowleri,
Acanthamoeba sp., Giardia lambia, Cryptosporidium sp.,
Pneumocystis carinii, Plasmodium vivax, Babesia microti,
10   Trypanosoma brucei, Trypanosoma cruzi, Leishmania donovani,
Toxoplasma gondi, and Nippostrongylus brasiliensis.

If the condition ameliorated by immune stimulation,
in particular stimulation of antigen-specific T-lymphocytes
is immunization against an antigen, the anti-human CD27
15   agonistic antibody and the number of immune checkpoint
inhibitors are in general administered in combination with a
vaccine. The vaccine may comprise a number of antigen or
antigenic determinants specific for, a pathogen, such as a
pathogen selected from bacteria, fungi, viruses or
20   parasites, or of a toxin, or a self-antigen, including
antigens expressed on benign or malignant tumors, such as
cancers. The pathogens and cancers against which the
vaccination is directed may be selected as indicated above.

According to certain embodiments the vaccination is
25   directed against HIV, Hepatitis (A, B, C, D, E), Influenza,
Herpes, Giardia, Malaria, Leishmania, Staphylococcus aureus,
or Pseudomonas Aeruginosa, preferably HIV. For these
pathogens there is currently no effective vaccine, or
existing vaccines are less than completely effective.
30   The inventors of the present invention have found
unexpected immune stimulatory effects when combining an
anti-human CD27 agonistic antibody and a number of immune
checkpoint protein inhibitors, in particular an inhibitor of

CTLA4 or PD1, PD-L1, PD-L2, LAG-3, BTLA, B7H3, B7H4, TIM3 or KIR. Tests indicate synergistic and/or potentiating effects of the combinations. Thus according to certain embodiments the anti-human CD27 agonistic antibody is for synergistic

5   and/or potentiating stimulation of the immune response, particularly synergistic and/or potentiating stimulation of antigen-specific T-lymphocytes, together with the number of immune checkpoint inhibitors.

The anti-human CD27 agonistic antibody of the

10   present invention preferably is presented in a composition comprising the antibody. The composition comprises the antibody or antibodies together with a carrier. The composition according to certain embodiments preferably is a pharmaceutical composition.

15   To prepare pharmaceutical or sterile compositions, the antibody, antibodies or fragment thereof, is admixed with a pharmaceutically acceptable carrier and/or excipient, see, e.g., Remington's Pharmaceutical Sciences and U.S. Pharmacopeia: National Formulary, Mack Publishing Company,

20   Easton, PA (1984). Formulations of therapeutic and diagnostic agents may be prepared by mixing with physiologically acceptable carriers, excipients, or stabilizers in the form of, e.g., lyophilized powders, slurries, aqueous solutions or suspensions (see, e.g.,

25   Hardman, *et al.*, 2001, *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, McGraw-Hill, New York, NY; Gennaro, 2000, Remington: *The Science and Practice of Pharmacy*, Lippincott, Williams, and Wilkins, New York, NY; Avis, *et al.* (eds.), 1993, *Pharmaceutical Dosage Forms:*

30   *Parenteral Medications*, Marcel Dekker, NY; Lieberman, *et al.* (eds.), 1990, *Pharmaceutical Dosage Forms: Tablets*, Marcel Dekker, NY; Lieberman, *et al.* (eds.), 1990, *Pharmaceutical Dosage Forms: Disperse Systems*, Marcel Dekker, NY; Weiner

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and Kotkoskie, 2000, *Excipient Toxicity and Safety*, Marcel
Dekker, Inc., New York, NY).

Toxicity and therapeutic efficacy of the binding
compound, in particular antibody, compositions, administered
5   alone or in combination with another agent, such as the
usual anti-cancer drugs, can be determined by standard
pharmaceutical procedures in cell cultures or experimental
animals, e.g., for determining the LD_{50} (the dose lethal to
50% of the population) and the ED_{50} (the dose therapeutically
10   effective in 50% of the population). The dose ratio between
toxic and therapeutic effects is the therapeutic index and
it can be expressed as the ratio between LD_{50} and ED_{50}. The
data obtained from these cell culture assays and animal
studies can be used in formulating a range of dosage for use
15   in humans. The dosage of such compounds lies preferably
within a range of circulating concentrations that include
the ED_{50} with little or no toxicity. The dosage may vary
within this range depending upon the dosage form employed
and the route of administration utilized.
20   Suitable routes of administration include
parenteral administration, such as intramuscular,
intravenous, or subcutaneous administration and oral
administration. Administration of antibodies, used in the
pharmaceutical composition or to practice the method of the
25   present invention can be carried out in a variety of
conventional ways, such as oral ingestion, inhalation,
topical application or cutaneous, subcutaneous,
intraperitoneal, parenteral, intraarterial or intravenous
injection. In one embodiment, the binding compound of the
30   invention is administered intravenously. In another
embodiment, the binding compound of the invention is
administered subcutaneously.

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Alternatively, one may administer the antibodies in a local rather than systemic manner, for example, via injection of the antibody or antibodies directly into the site of action, often in a depot or sustained release formulation. Furthermore, one may administer the antibody in a targeted drug delivery system.

Guidance in selecting appropriate doses of antibodies, cytokines, and small molecules are available (see, e.g., Wawrzynczak, 1996, *Antibody Therapy*, Bios Scientific Pub. Ltd, Oxfordshire, UK; Kresina (ed.), 1991, *Monoclonal Antibodies, Cytokines and Arthritis*, Marcel Dekker, New York, NY; Bach (ed.), 1993, *Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases*, Marcel Dekker, New York, NY; Baert, *et al.*, 2003, *New Engl. J. Med.* 348:601-608; Milgrom, *et al.*, 1999, *New Engl. J. Med.* 341:1966-1973; Slamon, *et al.*, 2001, *New Engl. J. Med.* 344:783-792; Beniaminovitz, *et al.*, 2000, *New Engl. J. Med.* 342:613-619; Ghosh, *et al.*, 2003, *New Engl. J. Med.* 348:24-32; Lipsky, *et al.*, 2000, *New Engl. J. Med.* 343:1594-1602).

Determination of the appropriate dose is made by the clinician, e.g., using parameters or factors known or suspected in the art to affect treatment or predicted to affect treatment. Generally, the dose begins with an amount somewhat less than the optimum dose and it is increased by small increments thereafter until the desired or optimum effect is achieved relative to any negative side effects. Important diagnostic measures include those of symptoms of, e.g., the inflammation or level of inflammatory cytokines produced.

A preferred dose protocol is one involving the maximal dose or dose frequency that avoids significant undesirable side effects. A total weekly dose is generally at least 0.05 μg/kg body weight, more generally at least 0.2

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µg/kg, most generally at least 0.5 µg/kg, typically at least
1 µg/kg, more typically at least 10 µg/kg, most typically at
least 100 µg/kg, preferably at least 0.2 mg/kg, more
preferably at least 1.0 mg/kg, most preferably at least 2.0

5   mg/kg, optimally at least 10 mg/kg, more optimally at least
25 mg/kg, and most optimally at least 50 mg/kg (see, e.g.,
Yang, *et al.*, 2003, *New Engl. J. Med.* 349:427-434; Herold,
et al., 2002, *New Engl. J. Med.* 346:1692-1698; Liu, *et al.*,
1999, *J. Neurol. Neurosurg. Psych.* 67:451-456; Portielji, *et*

10 *al.*, 2003, *Cancer Immunol. Immunother.* 52:133-144). The
desired dose of a small molecule therapeutic, e.g., a
peptide mimetic, natural product, or organic chemical, is
about the same as for an antibody or polypeptide, on a
moles/kg basis.

15   "Administration", "therapy" and "treatment," as it
applies to an animal, human, experimental subject, cell,
tissue, organ, or biological fluid, refers to contact of an
exogenous pharmaceutical, therapeutic, diagnostic agent, or
composition to the animal, human, subject, cell, tissue,

20 organ, or biological fluid. "Administration", "therapy" and
"treatment" can refer, e.g., to therapeutic,
pharmacokinetic, diagnostic, research, and experimental
methods. Treatment of a cell encompasses contact of a
reagent to the cell, as well as contact of a reagent to a

25 fluid, where the fluid is in contact with the cell.
"Administration", "therapy" and "treatment" also mean *in
vitro* and *ex vivo* treatments, e.g., of a cell, by a reagent,
diagnostic, binding composition, or by another cell.
        As used herein, "inhibit" or "treat" or

30 "treatment" includes a postponement of development of the
symptoms associated with disease and/or a reduction in the
severity of such symptoms that will or are expected to
develop with said disease. The terms further include

ameliorating existing symptoms, preventing additional
symptoms, and ameliorating or preventing the underlying
causes of such symptoms. Thus, the terms denote that a
beneficial result has been conferred on a vertebrate subject
5   with a disease.

As used herein, the term "therapeutically
effective amount" or "effective amount" refers to an amount
of antibody or antibodies, that when administered alone or
in combination with an additional therapeutic agent to a
10   cell, tissue, or subject is effective to prevent or
ameliorate the disease or condition to be treated. A
therapeutically effective dose further refers to that amount
of the compound sufficient to result in amelioration of
symptoms, e.g., treatment, healing, prevention or
15   amelioration of the relevant medical condition, or an
increase in rate of treatment, healing, prevention or
amelioration of such conditions. When applied to an
individual active ingredient administered alone, a
therapeutically effective dose refers to that ingredient
20   alone. When applied to a combination, a therapeutically
effective dose refers to combined amounts of the active
ingredients that result in the therapeutic effect, whether
administered in combination, serially or simultaneously. An
effective amount of therapeutic will decrease the symptoms
25   typically by at least 10%; usually by at least 20%;
preferably at least about 30%; more preferably at least 40%,
and most preferably by at least 50%.

Methods for co-administration or treatment with a
second therapeutic agent are well known in the art, see,
30   e.g., Hardman, *et al.* (eds.), 2001, *Goodman and Gilman's The
Pharmacological Basis of Therapeutics*, 10th ed., McGraw-
Hill, New York, NY; Poole and Peterson (eds.), 2001,
Pharmacotherapeutics for Advanced Practice: A Practical

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Approach, Lippincott, Williams & Wilkins, Phila., PA;
Chabner and Longo (eds.), 2001, *Cancer Chemotherapy and*
Biotherapy, Lippincott, Williams & Wilkins, Phila., PA.

Within the present invention the term 'co-

5   administered' should be understood as meaning that the
individual active components (here the anti-human CD27
agonistic antibody and the number of immune checkpoint
inhibitors) are administered in the same subject. Such
administration may be simultaneously or alternatively the

10   active components may be administered within a time frame of
up to 3 months, in view of the fact that most antibody
therapeutics, including those targeting immune checkpoint
inhibitors and CD27 display a terminal half-life in human
subjects of 2-4 weeks. Therefore, pharmacodynamic responses

15   to such antibody may be detectable for months after
administration.

The pharmaceutical composition of the invention
may also contain other agents, including but not limited to
a cytotoxic, chemotherapeutic, cytostatic, anti-angiogenic

20   or antimetabolite agents, a tumor targeted agent, an immune
stimulating or immune modulating agent or an antibody
conjugated to a cytotoxic, cytostatic, or otherwise toxic
agent. The pharmaceutical composition can also be employed
with other therapeutic modalities such as surgery,

25   chemotherapy and radiation.

According to a preferred embodiment the anti-human
CD27 agonistic antibody is provided, in a pharmaceutical
composition comprising the anti-human CD27 agonistic
antibody together with a pharmaceutically acceptable

30   carrier, said pharmaceutical composition being packed in a
container and said container being associated with an
information carrier, wherein said information carrier
comprises information indicating the combined use of the

anti-human CD27 agonistic antibody, preferably in the pharmaceutical composition, together with an immune checkpoint inhibitor for use in the treatment of a condition ameliorated by stimulation of an immune response, in

5   particular stimulation of antigen-specific T-lymphocytes. The container may be any container suitable for holding a pharmaceutical composition, preferably a sterile pharmaceutical composition, more preferably an injectable pharmaceutical composition. According to a preferred

10   embodiment the container comprises a unit dosage of the anti-human CD27 agonistic antibody.

The information carrier may be any suitable information carrier such as an object having a surface suitable for carrying information, for example paper or

15   cardboard. Alternatively a data carrier comprising machine readable information may be used. The information may be presented in visual form such as in the form of written information or in the form of a number of pictures. Alternatively the information may be presented in a form

20   "readable" by touch, such as in braille. In case the information is on a data carrier comprising machine readable information, the information may be stored as machine code or analogous signals which can be converted to visual and/or audio information.

25   The information carrier and the container comprising the pharmaceutical composition are associated such that they can be presented together in a single product. For example they may be associated by being enclosed together in an enclosure, such as a container,

30   including a box.

A further aspect of the invention relates to an immune checkpoint inhibitor for use in the treatment of a condition ameliorated by stimulation of an immune response,

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in particular the treatment of a condition that benefits
from the stimulation of antigen-specific T-lymphocytes,
wherein in said treatment an anti-human CD27 agonistic
antibody is administered. This immune checkpoint inhibitor

5   together with the anti-human CD27 agonistic antibody forms a
therapeutic combination resulting in unexpected immune
stimulation. According to certain embodiments the immune
checkpoint inhibitor is for synergistic and/or potentiating
stimulation of the immune response, particularly synergistic

10   and/or potentiating stimulation of antigen-specific T-
lymphocytes, together with the anti-human CD27 agonistic
antibody. The technical details of the various features and
preferred embodiments of the immune checkpoint inhibitor of
the invention is similar to what has already been discussed

15   in connection to the anti-human CD27 agonistic antibody of
the invention.

        Similar to the anti-human CD27 agonistic antibody,
the immune checkpoint inhibitor of the invention may be
provided, in a pharmaceutical composition comprising the

20   immune check point inhibitor together with a
pharmaceutically acceptable carrier, said pharmaceutical
composition being packed in a container and said container
being associated with an information carrier, wherein said
information carrier comprises information indicating the

25   combined use of the immune checkpoint inhibitor, preferably
in the pharmaceutical composition, together with an anti-
human CD27 agonistic antibody for use in the treatment of a
condition ameliorated by stimulation of an immune response,
in particular the treatment of a condition that benefits

30   from stimulation of antigen-specific T-lymphocytes. The
container may be any container suitable for holding a
pharmaceutical composition, preferably a sterile
pharmaceutical composition more preferably an injectable

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pharmaceutical. According to a preferred embodiment the
container comprises a unit dosage of the anti-human CD27
agonistic antibody. Also for this embodiment of the
invention the details of the various technical features and
5   preferred embodiments will be clear from the parts of the
description relating to the anti-human CD27 agonistic
antibody.

        Yet a further aspect of the invention relates to a
combination of an anti-human CD27 agonistic antibody
10   together with a number of immune checkpoint inhibitors for
use in the treatment of a condition ameliorated by
stimulation of an immune response, in particular the
treatment of a condition that benefits from the stimulation
of antigen-specific T-lymphocytes. This combination
15   according to the invention is primarily a functional
combination and any physical combination considered suitable
by the skilled person for making this functional combination
is to be considered within the scope of this invention. For
example in the combination the anti-human CD27 agonistic
20   antibody may be co-administered with the number of immune
checkpoint inhibitors to a subject. Alternatively, the
combination may be a kit of parts comprising:

        (i) a first container holding a first
pharmaceutical composition comprising the anti-human CD27
25   agonistic antibody together with a pharmaceutically
acceptable carrier;

        (ii) a second container holding a second
pharmaceutical composition comprising the immune checkpoint
inhibitor together with a pharmaceutically acceptable
30   carrier

        (iii) optionally an information carrier comprising
information indicating the combined use of the anti-human
CD27 agonistic antibody, preferably in the first

pharmaceutical composition, together with an immune checkpoint inhibitor, preferably in the second pharmaceutical composition, for use in the treatment of a condition ameliorated by stimulation of an immune response,

5   in particular the treatment of conditions that benefit from the stimulation or enhancement of antigen-specific T-lymphocytes.

        According to an alternative embodiment the first and second container and the first and second pharmaceutical

10   composition coincide thus presenting a kit of parts comprising:

        (A) a container holding a pharmaceutical composition comprising the anti-human CD27 agonistic antibody and the immune checkpoint inhibitor together with a

15   pharmaceutically acceptable carrier;

        (B) optionally an information carrier comprising information indicating the combined use of the anti-human CD27 agonistic antibody, preferably in the pharmaceutical composition, together with an immune checkpoint inhibitor,

20   preferably in the pharmaceutical composition, for use in the treatment of a condition ameliorated by stimulation of an immune response, in particular the treatment of a condition that benefits from the stimulation of antigen-specific T-lymphocytes.

25   Also for the combination of the invention the details of the various technical features and preferred embodiments is similar to what has been discussed in the parts of the description relating to the anti-human CD27 agonistic antibody.

30   A further aspect of the invention relates to a method for treating a condition ameliorated by stimulation of an immune response, in particular the treatments of a condition that result in the stimulation of antigen-specific

T-lymphocytes, said method comprising administering a CD27 agonist, preferably an anti-human CD27 agonistic antibody, in combination with a number of immune checkpoint inhibitors.

5   Also for the method of treatment of the invention the details of the various technical features and preferred embodiments are similar to what has been discussed above in relation to the anti-human CD27 agonistic antibody.

          The invention and its effects will now be further
10   illustrated with reference to the following non-limiting examples.

                              EXAMPLES

EXAMPLE 1

15   *CD27 agonism combined with checkpoint protein blockade*
results in unexpected immune stimulation

          To study the effect of combining CD27 agonists with PD-1 or PD-L1 blocking antibodies human Peripheral Blood Mononuclear cells (PBMNCs) were isolated from buffy
20   coat. First, the Buffy coat was diluted to a total volume of 300 ml with DMEM/F12 medium (Gibco, 11320) supplemented with heparin solution (Leo Pharma, DB6132, 5000 U/ml) at RT. After mixing the cell suspension, aliquotes were loaded on a Ficoll-Paque Plus gradient in conical tubes and centrifuged
25   at 450 g for 30 min, at 20°C without a brake. Next, plasma was removed by aspiration and PBMCs were recovered from the plasma/Ficoll interface. PBMCs were washed twice with DMEM/F12 medium and resuspended in RPMI 1640 medium (Gibco, 52400) supplemented with 10% Foetal Calf Serum. PBMCs were
30   plated at 2×10^5 cells/well in 96-well flat bottom plates (Nuclon). Antibodies (anti-PD1 (hIgG4 chimera, see below, of hPD109A, WO/2008/156712), anti-hCD27 (hIgG4 chimera of hCD27.15). See below for chimerization), anti-PDL1 (MIH1,

eBioscience 16.5983.82), human IgG4 isotype control (Sigma,
14639) and mouse IgG1 isotype control (eBioscience,
16.4714.85)) and dilutions thereof were diluted in PBS and
added to the PBMCs. Human IgG4 chimeric versions of hPD1.09A
5   and hCD27.15 were constructed by cloning of the VH and VL
genes upstream of the human IgG4 and human Kappa constant
domain encoding cDNA, respectively. Full cDNAs were
subcloned in pcDNA3.1 (Invitrogen) and transiently
transfected in 293T/17 cells (ATCC) using Lipofectamine 2000
10   (Invitrogen) following the manufacturer's instructions.
After 5-7 days, supernatants were harvested and antibodies
were purified using standard protein A purification.
Finally, Staphylococcus Enterotoxin B (Sigma S4881) diluted
in RPMI 1640 medium supplemented with 10% Foetal Calf Serum
15   was added to the wells in a final concentration of 25 ng/ml.
Cells were incubated for two days at 37°C, 5% CO_2 and 95%
humidity. To assess the level of immune activation, IL-2
secretion levels were determined in the supernatant in
accordance with the method described in Dulos *et al.*, *J.*
20   *Immunother*, 2012, 35:169-78. To that aim, supernatants were
aspirated and cleared from any cell material by
centrifugation. Next, supernatants were added to Nunc
maxisorp ELISA plates that had been coated with 2 µg/ml
anti-hIL-2 antibody (BDPharmingen, 555051) in PBS by
25   overnight incubation at 4°C. Prior to addition of the
supernatants, wells were emptied and blocked with 200
µl/well PBS/1%BSA for one hour at Room Temperature (RT).
Supernatants were incubated for one hour at RT, washed three
times with PBST (PBS with 0.01% Tween 20). Subsequently, 100
30   µl of 0.5 µg/ml of anti-hIL2-biotin (BD Pharmingen 555040)
was added in PBS/PBS-1%BSA (1:1) and incubated for one hour
at RT. After three washes with PBST, 1:5000 diluted
streptavidin-HRP (BD Pharmingen, 554066) was added in 100 µl

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PBS/PBS-1% BSA (1:1). After three washes with PBST and one
wash with water, IL-2 was detected by addition of 100
µl/well TMB stabilized chromogen (Invitrogen, SB02).
Reactions were stopped with 100 µl 0.5 M H_2SO_4 and
5   absorbances were read at 450 and 620 nm. In this assay,
recombinant human IL-2 (Sigma, H7041) was used to quantify
IL-2 protein levels in the supernatants. In **Figure 1A** the
cooperative action between CD27 agonistic antibody and PD-1
blocking antibody and in **Figure 1B** the cooperative action
10 between CD27 agonistic antibody and PD-L1 blocking antibody
is shown.

        To study the effect of combining CD27 agonist with
PD-1 and PD-L1 blocking antibody in whole human blood, blood
was diluted 10 times in RPMI 1640 medium (Gibco, 52400)
15 supplemented with 10% Foetal Bovine Serum (Hyclone). Diluted
blood was plated in 96-well Nunclon delta surface flat
bottom plates (100 µl/well). Antibodies anti-hCD27 (hIgG4
chimera of hCD27.15, see above)), anti-PD-1 (hIgG4 chimera
of hPD1.09A, see above), anti-PDL1 (MIH1, eBioscience
20 16.5983.82), human IgG4 isotype control (Sigma, 14639) and
mouse IgG1 isotype control (eBioscience, 16.4714.85) and
dilutions thereof were diluted in PBS and added to the
diluted blood. Finally, Staphylococcus Enterotoxin B (Sigma
S4881) diluted in RPMI 1640 medium supplemented with 10%
25 Foetal Calf Serum was added to the wells in a final
concentration of 25 ng/ml. Cells were incubated for two days
at 37°C, 5% CO_2 and 95% humidity. To assess the level of
immune activation, IL-2 secretion levels were determined in
the supernatant. To that aim, supernatants were aspirated
30 and cleared from any cell material by centrifugation. Next,
supernatants were added to Nunc maxisorp ELISA plates that
had been coated with 2 µg/ml anti-hIL-2 antibody (BD
Pharmingen, 555051) in PBS by overnight incubation at 4°C.

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Prior to addition of the supernatant, wells were emptied and blocked with 200 µl/well PBS/1%BSA for one hour at Room Temperature (RT). Supernatants were incubated for one hour at RT, washed three times with PBST (PBS with 0.01% Tween

5   20). Subsequently, 100 µl of 0.5 µg/ml of anti-hIL2-biotin (BD Pharmingen 555040) was added in PBS/PBS-1%BSA (1:1) and incubated for one hour at RT. After three washes with PBST, 1:5000 diluted streptavidin-HRP (BD Pharmingen, 554066) was added in 100 µl PBS/PBS-1% BSA (1:1). After three washes

10   with PBST and one wash with water, IL-2 was detected by addition of 100 µl/well TMB stabilized chromogen (Invitrogen, SB02). Reactions were stopped with 100 µl 0.5 M H_2SO_4 and absorbances were read at 450 and 620 nm. In this assay, recombinant human IL-2 (Sigma, H7041) was used to

15   quantify IL-2 protein levels in the supernatants. Also in this test the IL-2 level was increased to an unexpected level by the combination of the CD27 agonistic antibody and the PD-1 or PDL-1 inhibiting antibody in comparison to these antibodies alone.

20   The expectation presented by these results, that combinations of a CD27 agonistic antibody with other immune check point inhibitors will give similar effects, was confirmed in additional experiments performed with the same whole blood test protocol, which included the combination of

25   a CD27 agonist (hIgG4 chimera of hCD27.15, see above) with anti-CTLA4 (14D3, eBioscience 16.1529.82). The results of these experiments (see **Figures 2A-2C**) showed a similar cooperative action by the combination of the CD27 agonistic antibody and the CTLA4 inhibiting antibody in comparison to

30   the individual antibodies alone (IgG4 isotype control as above; IgG2A isotype control: BD Pharmingen 554126).

          On the basis of these results it may be expected that the combination of an anti-human CD27 agonistic

antibody together with at least one immune checkpoint
inhibitor will have beneficial effects in conditions
ameliorated by stimulation of an immune response, such as
conditions ameliorated by stimulation of antigen-specific T-
5   lymphocytes.

Example 2

*Evaluating the activity of anti-CD27, anti-LAG-3, and anti-
PD1 antibodies in a human PBMC SEB superantigen-induced IL2*
10 *secretion*

An additional confirmatory experiment was conducted
to further show the effects of the combination of anti-human
CD27 agonistic antibodies with immune checkpoint protein
inhibitors. In this experiment a hCD27.15 analogue was used
15 in combination with an anti-LAG3 antibody and an anti-PD1
antibody. The analogue used contains functional heavy chain
CDR1, CDR2, CDR3 (SEQ ID NO: 1, 2, 3, respectively) and
light chain CDR1, CDR2, CDR3 (SEQ ID NO: 4, 5, 6,
respectively) of hCD27.15. In addition it contains
20 functionally linked functional human IgG4 constant domains
(CH1 – CH3, GenBank accession #K01316). Functionality of the
hCD27.15 analogue was established in a CD27 binding assay
corresponding to the one disclosed in example 2 (page 49-50)
of WO2012/004367 and a CD27 signaling test corresponding to
25 the NF-κB tests disclosed in example 3 (page 54-55) of
WO2012/004367.

The human anti-LAG3 antibody used comprised a heavy
chain amino acid sequence according to SEQ ID NO: 23 and a light
chain amino acid sequence according to SEQ ID NO: 24. The human
30 anti-PD1 antibody used comprised a heavy chain amino acid
sequence according to SEQ ID NO: 21 and a light chain amino acid
sequence according to SEQ ID NO: 22. To study the effect of the
combination of anti-human CD27 agonistic antibodies with

immune checkpoint protein inhibitors human PBMCs were
isolated from heparinized human blood from heathy donors
using SepMate® tubes (Stem Cell). 15 ml of Ficoll-1077 was
added to SepMate® tubes, which was carefully overlayed with
5   25 ml of human blood and centrifuged at 1,250 g for 12 min.
at RT with light brake (5 out of 10 – 10 being max brake).
White blood cells were isolated at the interphase of the
Ficoll and dilute into 40 ml of Hanks Balances Salt Solution
(HBSS) at RT. Next, cells were centrifuged at 300 g for 10
10   min. at 4 °C and the pellet was resuspended in 50 ml of HBSS.
Finally, the cell suspension was centrifuged at 250 g for 10
min to remove platelets and the pellet was resuspended in 12
ml complete media (RPMI 1640 containing HEPES and Penn/Step
and 10% human A+B+ serum)). Cells were quantified by Vi-
15   cell. Next, 50 µl of PBMC cell suspension (1 x 10^7 cells/ml
(final 5 x 10^5 cells/well) were plated in a 96-well round
bottom plate. 50 µl of antibody (per antibody treatment, so
100 µl total antibody added for combination conditions) at
10 µg/ml end concentrations for 30 min. at 37°C. Next, a 4x
20   concentration of 50 µl *Staphyloccus* enterotoxin B (SEB)
superantigen (Toxin Technology, Sarasota, FL) at 0.5, 1, 8,
16, 32, 64 and 80 ng/ml end concentration was added and
incubate 72 hr at 37°C. Supernatants were collected and
cleared from any cell material by centrifugation. IL-2
25   secretion levels as a measure for immune activation was
detected using Human IL-2 V-PLEX Kit (Catalog No. K151QQD-4,
from Meso Scale Discovery, Rockville, MD), according to the
manufacturer's instructions. As a negative control, (sub)-
isotype matched antibodies were used.

30   **Figures 3A-3D** show the results obtained from four
separate donors using the experimental methods described
above. For each donor the results of the anti-CD27/Anti-LAG3
combination is shown in the left panel and the results of

the anti-CD27/Anti-PD1 combination is shown in the right
panel. For each SEB concentration the bars represent from
left to right: isotype control; immune checkpoint inhibitor
(anti-Lag3 or anti-PD1); anti-CD27; immune checkpoint

5   inhibitor (anti-Lag3 or anti-PD1) + anti-CD27. The anti-CD27
antibody demonstrates approximately 1.5-2 fold IL-2 increase
above isotype controls as a single agent. The anti-CD27
antibody showed combination activity with anti-LAG-3
antibody and with anti-PD1 antibody. Similar effects may be

10   expected for combinations of anti-human CD27 agonistic
antibody (including analogues) with other immune checkpoint
protein inhibitors.

CLAIMS

1. An anti-human CD27 agonistic antibody, such as hCD27.15
   or 1F5, or an antibody analogue thereof, for use in the
   treatment of a condition ameliorated by stimulation of
   an immune response, in particular stimulation of
   antigen-specific T-lymphocytes, wherein in said
   treatment a number of immune checkpoint protein
   inhibitors is administered.

2. An anti-human CD27 agonistic antibody according to
   claim 1, wherein an immune checkpoint protein inhibitor
   is selected from an inhibitor of CTLA-4, PD1, PD-L1,
   PD-L2, LAG-3, BTLA, B7H3, B7H4, TIM3 or KIR.

3. An anti-human CD27 agonistic antibody according to any
   of the claims 1-2, wherein the condition ameliorated by
   immune stimulation, in particular stimulation of
   antigen-specific T-lymphocytes is selected from
   infectious diseases, such as bacterial, fungal, viral
   and parasitic infectious diseases, immunization against
   a pathogen, such as a pathogen selected from bacteria,
   fungi, viruses or parasites, or vaccination against
   toxins, or self-antigens, including antigens expressed
   on benign or malignant tumors, such as cancers, or
   conditions associated with uncontrolled proliferation
   of cells such as cancers.

4. An anti-human CD27 agonistic antibody according to any
   of the claims 1-3, wherein the treatment is vaccination
   and a vaccine is administered in the treatment.

5. Immune checkpoint inhibitor for use in the treatment of
   a condition ameliorated by stimulation of an immune
   response, in particular stimulation of antigen-specific
   T-lyphocytes, wherein in said treatment an anti-human

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CD27 agonistic antibody, such as hCD27.15 or 1F5, or an antibody analogue thereof, is administered.

6. Immune checkpoint inhibitor according to claim 5, wherein the immune checkpoint inhibitor is selected from an inhibitor of CTLA-4, PD1, PD-L1, PD-L2, LAG-3, BTLA, B7H3, B7H4, TIM3 or KIR.

7. Immune checkpoint inhibitor according to any of the claims 5-6, wherein the condition ameliorated by immune stimulation is selected from infectious diseases, such as bacterial, fungal, viral and parasitic infectious diseases, immunization against a pathogen, such as a pathogen selected from bacteria, fungi, viruses or parasites, or vaccination against toxins, or self-antigens, including antigens expressed on benign or malignant tumors, such as cancers, or conditions associated with uncontrolled proliferation of cells such as cancers.

8. An immune checkpoint inhibitor according to any of the claims 5-7, wherein the treatment is vaccination and wherein a vaccine is administered in the treatment.

9. Combination of an anti-human CD27 agonistic antibody, such as hCD27.15 or 1F5, or an antibody analogue thereof, together with a number of immune checkpoint inhibitors for use in the treatment of a condition ameliorated by stimulation of an immune response, particularly stimulation of antigen-specific T-lymphocytes.

10. Combination according to claim 9, wherein an immune checkpoint inhibitor is selected from an inhibitor of CTLA-4, PD1, PD-L1, PD-L2, LAG-3, BTLA, B7H3, B7H4, TIM3 or KIR.

11. Combination according to any of the claims 9-10, wherein the condition ameliorated by immune stimulation

is selected from infectious diseases, such as
bacterial, fungal, viral and parasitic infectious
diseases, immunization against a pathogen, such as a
pathogen selected from bacteria, fungi, viruses or
5   parasites, or vaccination against toxins, or self-
antigens, including antigens expressed on benign or
malignant tumors, such as cancers, or conditions
associated with uncontrolled proliferation of cells
such as cancers.

10 12. Combination according to any of the claims 9-11,
wherein the treatment is vaccination and wherein a
vaccine is administered in the treatment.

13. A method of treating a condition ameliorated by
stimulation of an immune response, comprising
15   administering to a subject in need thereof a
therapeutically effective amount of an anti-human CD27
agonistic antibody and further an immune checkpoint
protein inhibitor.

14. The method of claim 13, wherein said anti-human CD27
20   agonistic antibody is selected from the group
consisting of: an anti-human CD27 agonistic antibody
comprising the CDR amino acid sequences of SEQ ID NO:
1, 2, 3, 4, 5, 6, or a variant sequence; a humanized
analogue of antibody hCD27.15; an analogue of antibody
25   hCD27.15 that binds to the same epitope as hCD27.15;
antibody 1F5; an anti-human CD27 agonistic antibody
that does not require cross-linking.

15. The method of any of the claims 13-14, wherein said
further immune checkpoint inhibitor protein is selected
30   from the group consisting of: an CTLA-4 antibody, an
anti-PD1 antibody, an anti-PD-L1 antibody, an anti-PD-
L2 antibody, an anti LAG-3 antibody, an anti-BTLA

antibody, an anti-B7H3 antibody, an anti-B7H4 antibody, an anti-TIM3 antibody and an anti-KIR antibody.

16. The method of any of the claims 13-15, wherein said further immune checkpoint inhibitor protein is an anti-PD1 antibody.

17. The method of any of the claims 13-16, wherein said anti-PD-1 antibody is pembrolizumab.

18. The method of any of the claims 13-17, wherein said anti-PD-1 antibody is nivolumab.

19. The method of any of the claims 13-18, wherein said further immune checkpoint inhibitor protein is an anti-LAG3 antibody.

20. The method of claim 19, wherein said anti-LAG3 antibody comprises the heavy chain and light chain amino acid sequences of SEQ ID NO: 23 and SEQ ID NO: 24, respectively.

21. The method of any of the claims 13-20, wherein the subject in need of treatment suffers from cancer.

22. The method of any of the claims 13-20, wherein the subject in need of treatment suffers from an infection (such as a bacterial, fungal, viral and parasitic infectious diseases).

23. A vaccine comprising an anti-human CD27 agonistic antibody and further comprising an immune checkpoint protein inhibitor.

24. The vaccine of claim 23, wherein said further immune checkpoint protein inhibitor is selected from the group consisting of: an CTLA-4 antibody, an anti-PD1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti LAG-3 antibody, an anti-BTLA antibody, an anti-B7H3 antibody, an anti-B7H4 antibody, an anti-TIM3 antibody and an anti-KIR antibody.

25. The vaccine of any of the claims 23-24, wherein said further immune checkpoint inhibitor protein is an anti-PD1 antibody.

26. The vaccine of any of the claims 23-25, wherein said anti-PD-1 antibody is pembrolizumab.

27. The vaccine of claims 23-26, wherein said anti-PD-1 antibody is nivolumab.

28. The vaccine of any of the claims 23-27, wherein said further immune checkpoint inhibitor protein is an anti-LAG3 antibody.

29. The vaccine of any of the claims 23-28, wherein said anti-LAG3 antibody comprises the heavy chain and light chain amino acid sequences of SEQ ID NO:23 and SEQ ID NO:24, respectively.

## IL2 secretion

FIG. 1A

IL2 secretion
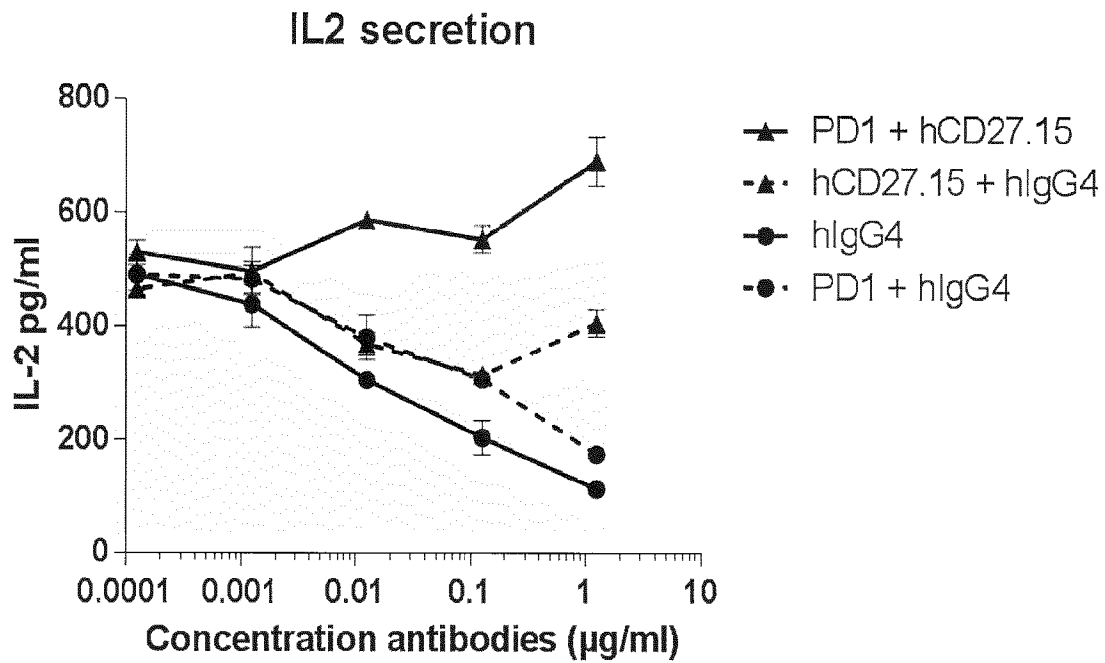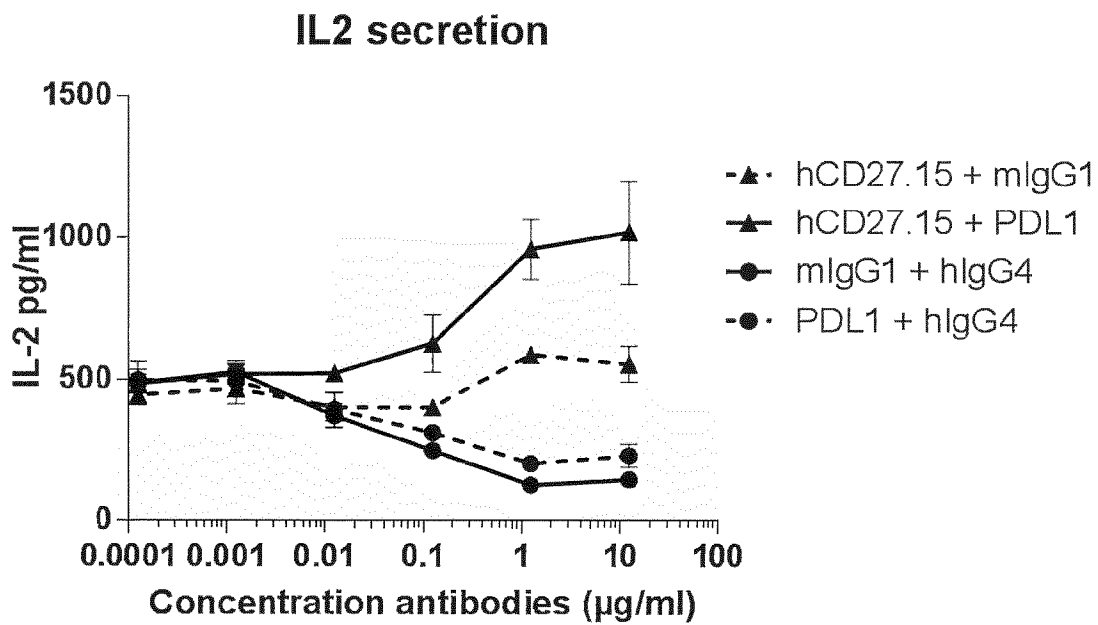
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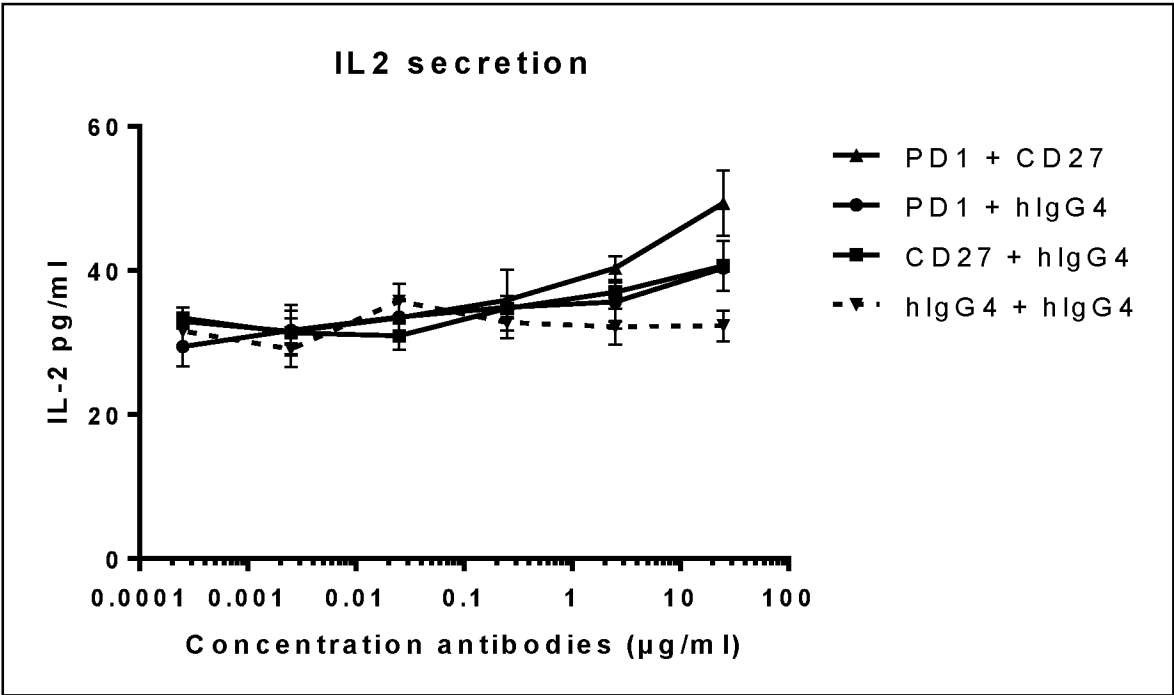
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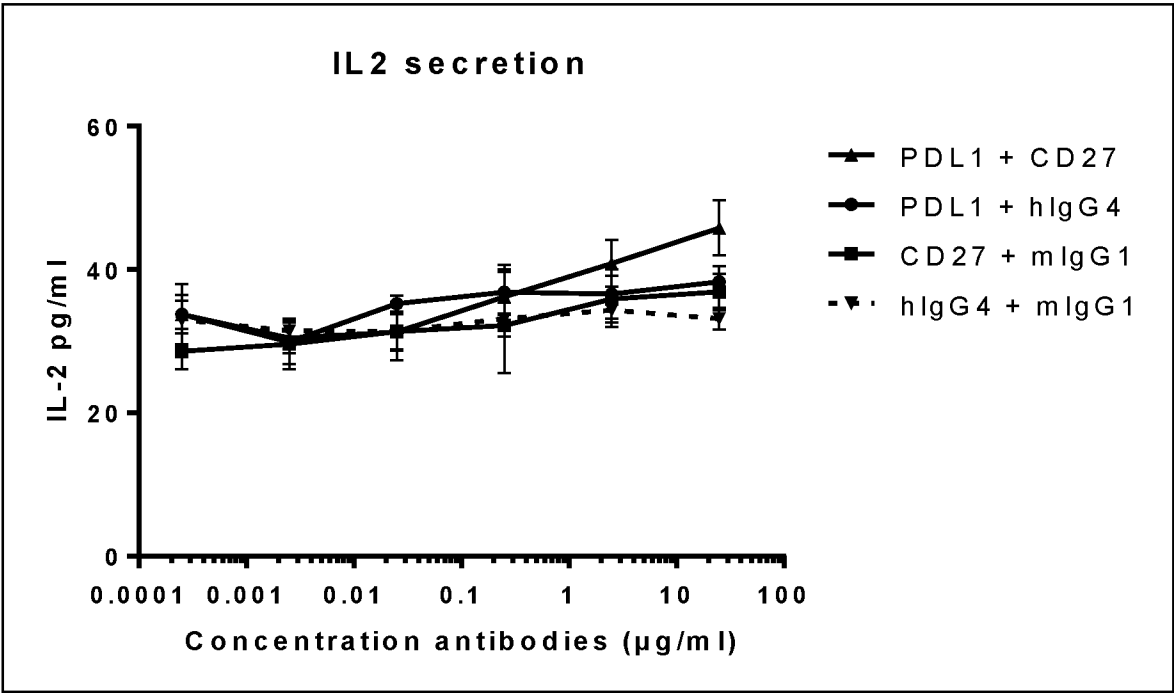
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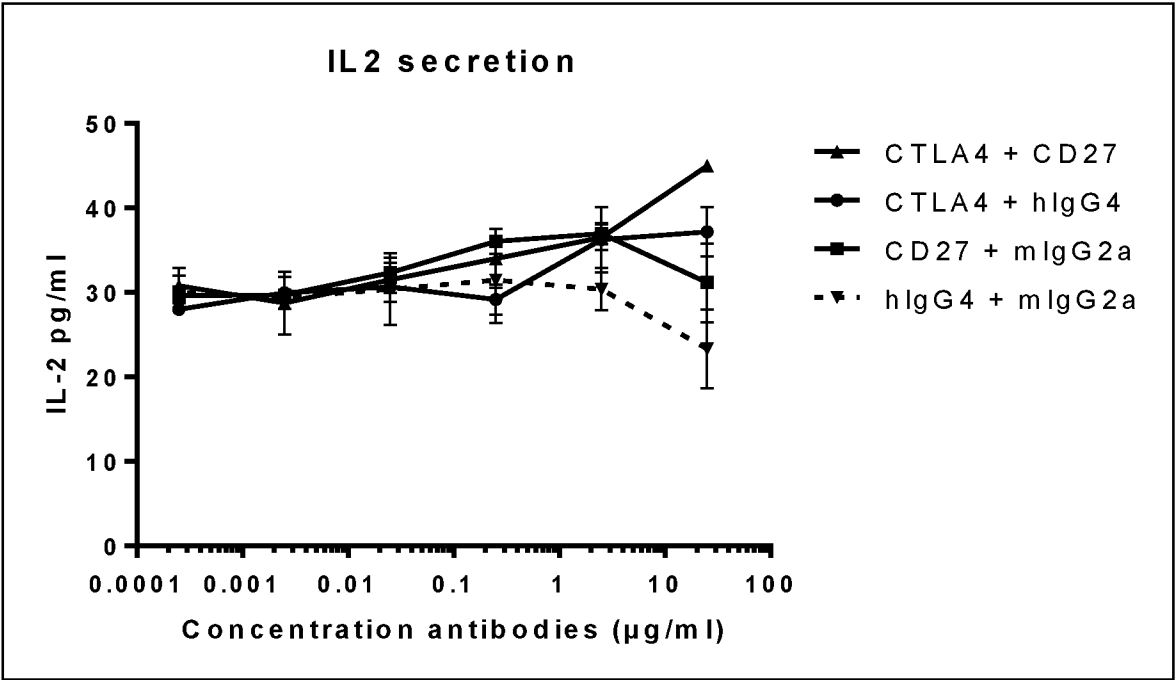
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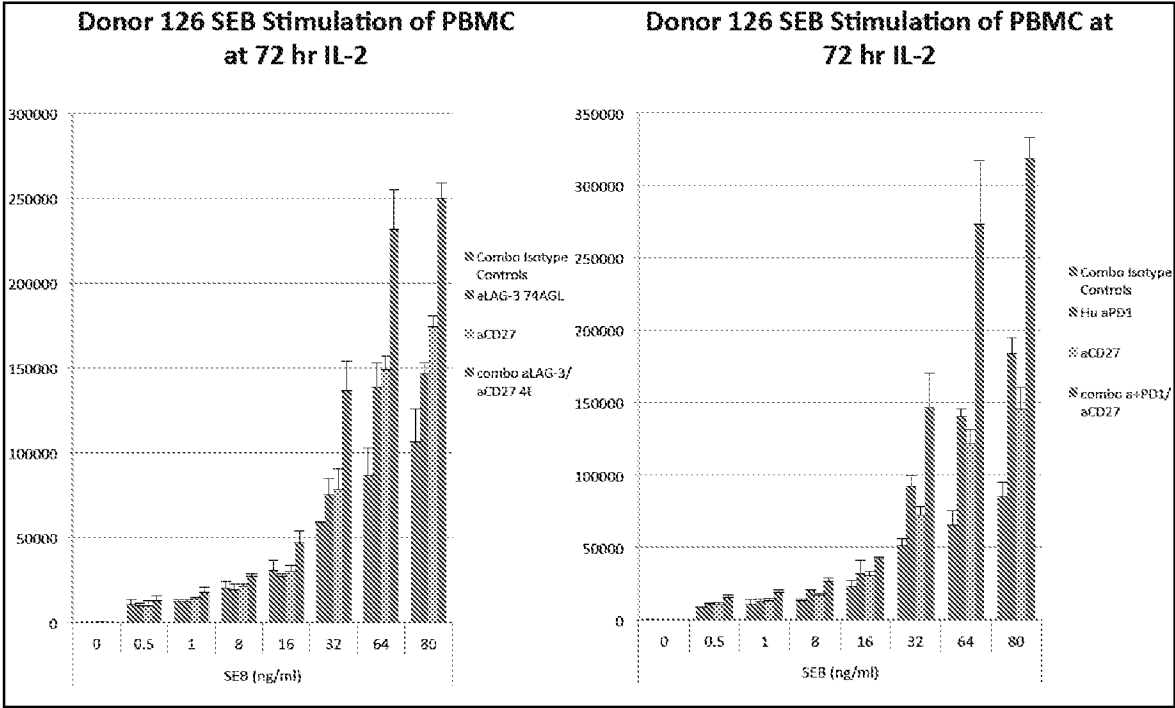
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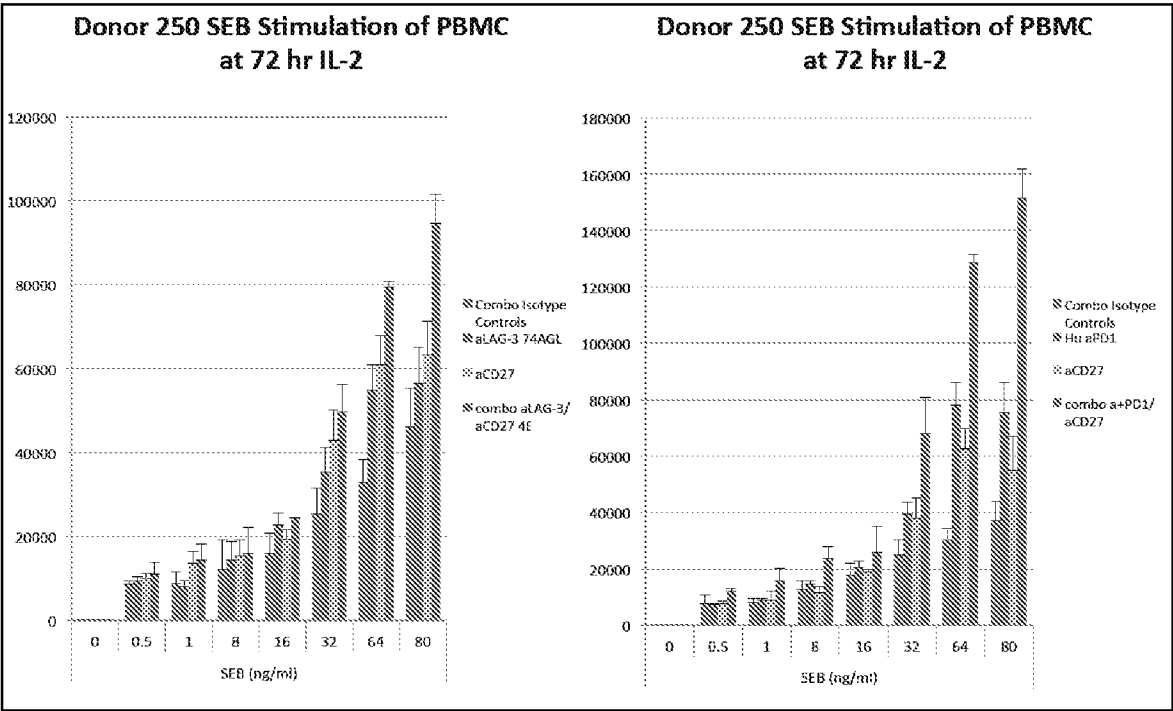
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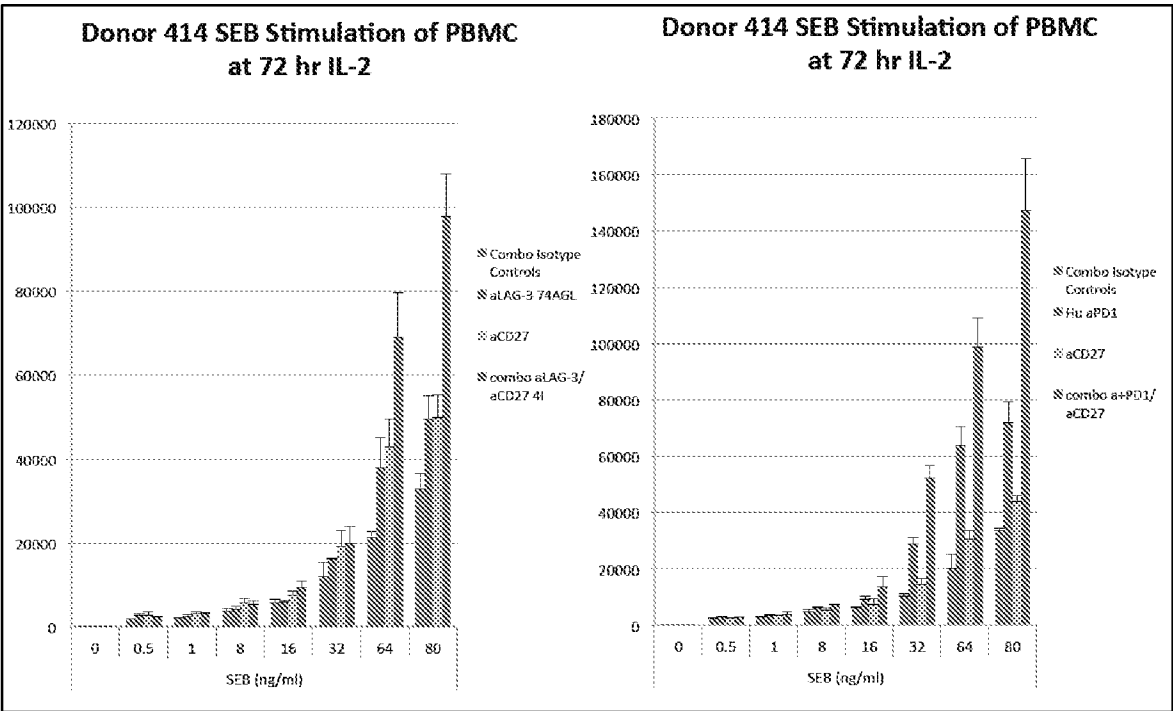
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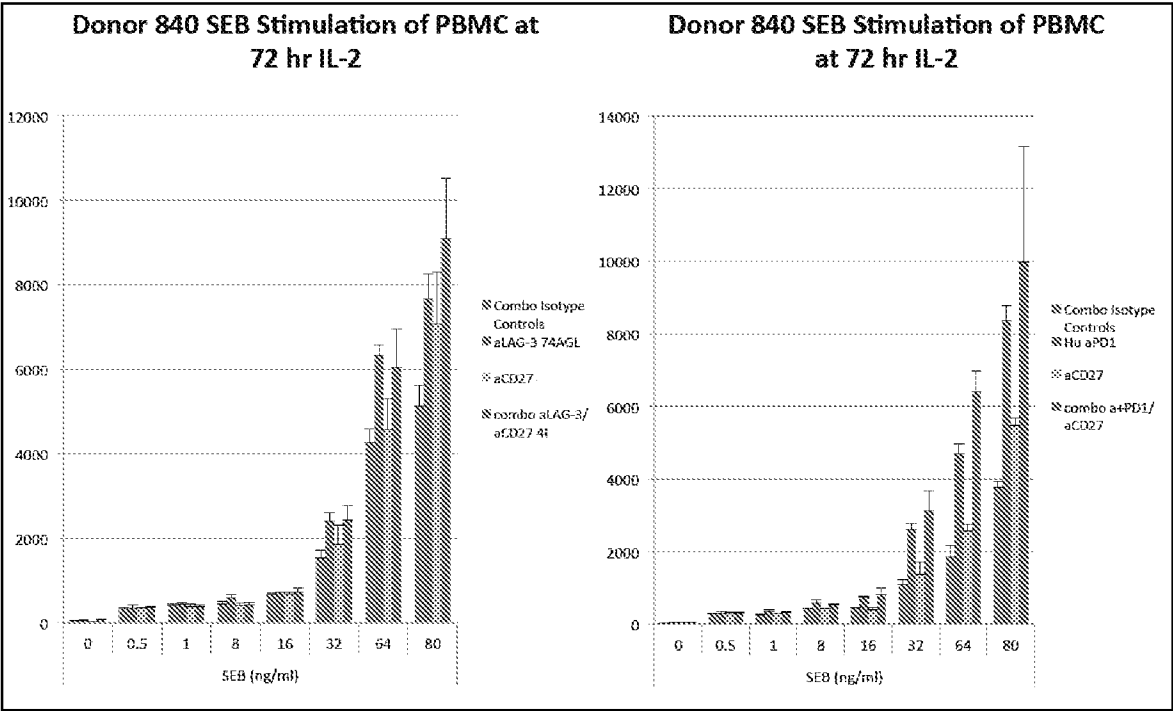
FIG. 3D

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Ser Leu Arg Leu Asp Cys Lys Ala Ser Gly Ile Thr Phe Ser Asn Ser
       20   25   30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
       35   40   45
Ala Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val
       50   55   60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65   70   75   80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
            85   90   95
Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
          100   105   110
Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser
       115   120   125
Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
       130   135   140
Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
145   150   155   160

```
Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
                165             170                 175
Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys
            180             185             190
Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp
            195             200             205
Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala
        210             215             220
Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
225             230             235             240
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
            245             250             255
Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
            260             265             270
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
        275             280             285
Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
290             295             300
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
305             310             315             320
Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
            325             330             335
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr
            340             345             350
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
            355             360             365
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
    370             375             380
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
385             390             395             400
Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
            405             410             415
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
            420             425             430
Ser Leu Ser Leu Ser Leu Gly Lys
            435             440
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<210> 22
<211> 214
<212> PRT
<213> Homo sapiens

<400> 22
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Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1               5                   10                  15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
            20              25              30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
            35              40              45
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50              55              60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65              70              75              80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Ser Asn Trp Pro Arg
            85              90              95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100             105             110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
            115             120             125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130             135             140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145             150             155             160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
            165             170             175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180             185             190
```

```
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205
Phe Asn Arg Gly Glu Cys
    210
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<210> 23
<211> 447
<212> PRT
<213> Artificial Sequence

<220>
<223>

<400> 23
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Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1                   5                   10                  15
Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Asp Tyr
                20                  25                  30
Tyr Trp Asn Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
            35                  40                  45
Gly Glu Ile Asn His Arg Gly Ser Thr Asn Ser Asn Pro Ser Leu Lys
        50                  55                  60
Ser Arg Val Thr Leu Ser Leu Asp Thr Ser Lys Asn Gln Phe Ser Leu
65                  70                  75                  80
Lys Leu Arg Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
                85                  90                  95
Phe Gly Tyr Ser Asp Tyr Glu Tyr Asn Trp Phe Asp Pro Trp Gly Gln
                100                 105                 110
Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
            115                 120                 125
Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
        130                 135                 140
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175
Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190
Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys
        195                 200                 205
Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro
        210                 215                 220
Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val
225                 230                 235                 240
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255
Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
                260                 265                 270
Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
            275                 280                 285
Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
        290                 295                 300
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320
Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
                325                 330                 335
Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
                340                 345                 350
Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
            355                 360                 365
Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
        370                 375                 380
Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400
Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
                405                 410                 415
Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
                420                 425                 430
```

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
          435   440   445

<210> 24
<211> 214
<212> PRT
<213> Artifical Sequence

<220>
<223>

<400> 24
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1   5   10   15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr
                20   25   30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
            35   40   45
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
        50   55   60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65   70   75   80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Leu
                85   90   95
Thr Phe Gly Gln Gly Thr Asn Leu Glu Ile Lys Arg Thr Val Ala Ala
            100   105   110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
            115   120   125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130   135   140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145   150   155   160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165   170   175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180   185   190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
            195   200   205
Phe Asn Arg Gly Glu Cys
    210