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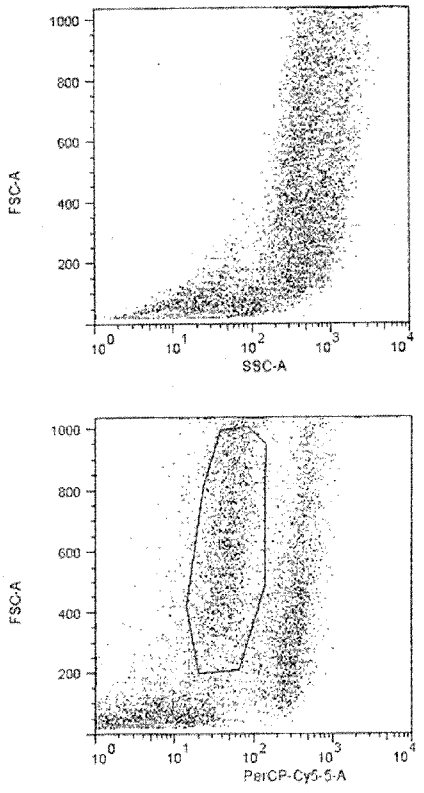
WO 2014/186878 A1

(54) Title: CELL SENESCENCE MARKERS AS DIAGNOSTIC AND THERAPEUTIC TARGETS

Fig. 1

HUVEC senescence staining with Alexa-488 (green fluorescence)



(57) Abstract: Provided are methods and agents for depleting senescent cells endogenous to a subject, involving administering to the subject a binding agent that is selectively toxic to senescent cells in an amount effective to reduce the number of such cells, wherein the binding agent binds selectively to a senescent cell surface protein having a misfolded conformation, relative to said protein in a native conformation.

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Cell Senescence Markers as Diagnostic and Therapeutic Targets

Related Applications

This is a Patent Cooperation Treaty Application which claims the benefit of priority of U.S. Provisional Patent Application No. 61/827,255, filed May 24, 2013 which is incorporated herein by reference in its entirety.

Field of the Invention

This invention relates to markers of cell senescence, and to applications thereof as targets for medical diagnosis and therapeutic intervention. More particularly, the invention relates to surface proteins that manifest in senescent cells, and to agents such as antibodies that bind such surface proteins for use in diagnosing and treating related disease conditions.

Background to the Invention

Senescence is generally defined as a cessation of cell replication that follows after a finite number of population doublings. Senescent cells remain metabolically active but do not divide and resist apoptosis for long periods. Cellular senescence is characterized by growth cycle arrest in the G1 phase, absence of S phase and lifespan control by multiple dominant genes.

Cellular senescence differs from quiescence and terminal differentiation. Senescent cells manifest morphological changes such as enlargement, flattening and increased granularity. They do not divide even if stimulated by mitogens. Replicative senescence is related to the progressive shortening of telomeres. Senescence is induced when certain chromosomal telomeres reach a critical length, and can be abrogated by the expression of telomerase which lengthens telomeres. Human fibroblasts, for instance, undergo replication indefinitely when transfected to express telomerase. Evidence suggests some relationship between replicative senescence and aging.

There are pathways to senescence other than replication. These are often referred to as stress-induced premature senescence (SIPS). Oxidative stress can shorten telomeres, and hyperoxia has been shown to induce senescence. Gamma irradiation of human fibroblasts in early to mid G1 phase causes senescence in a p53-dependent manner, and UV radiation also induces cellular senescence. Other agents that can induce cellular senescence include hydrogen peroxide, sodium butyrate, 5-azacytidine, and transfection with the Ras oncogene. Senescence in cancer cells can be induced by such chemotherapeutics as doxorubicin, cisplatin, and 5-bromodeoxyuridine, the latter being an agent that induces senescence in both normal and cancer cells.

The existence of cellular senescence has been demonstrated *in vivo*. Senescent fibroblasts were shown to exhibit staining for β-galactosidase activity at pH 6. These cells failed to incorporate tritiated thymidine and retained B-galactosidase activity after replating but did not divide. Quiescent fibroblasts did not show staining. Keratinocytes, umbilical vein endothelial cells and mammary epithelial cells all showed increased staining with increased population doublings. Immortalized cells and terminally differentiated keratinocytes did not show staining. Staining was performed on skin biopsies to test whether senescence is observed in vivo. An age-dependent pattern emerged in which an increased number of cells showed staining with increase donor age, in the dermis and epidermis. The existence of an increase in the number of senescent fibroblasts has been shown in the lungs of subjects with emphysema.

Cancer stem cells are immortal, meaning that they can replicate indefinitely without exhibiting senescence. Likely, the teleological purpose of senescence is to prevent cancer by limiting the number of cell divisions that can occur. Senescent cells can also promote tumorigenesis. Senescent stromal cells express tumour promoting as well as tumour suppressing factors that exert an effect on neighbouring epithelial cells, such as mitogenicity and anti-apoptosis.

In a dramatic demonstration, Baker et al report that a variety of age-related conditions were significantly improved in mice when senescent cells were removed (Nature, 2011, 479:232-236). Treated mice had substantially delayed lordokyphosis (a measure of sarcopenia) and cataracts, as well as increased muscle fiber diameter, reduced loss of adipose tissue, and improved skin tone. Some improvements were seen not only in younger mice, but also in older recipients in which age-related conditions were already established.

In summary, cellular senescence does occur *in vivo* and is a likely sequel to environmental insults. Its prevalence increases with age at least in some tissue compartments. Senescence confers functional changes on the cell which have been associated with various age-related diseases that include degenerative disorders such as Alzheimer's disease, cardiovascular disease, emphysema, sarcopenia, and tumorigenesis as well as conditions more cosmetic in nature such as signs of skin aging including wrinkling, sagging, discolouration, and the like. Senescent cells also develop a so-called senescence associated secretory phenotype (SASP) which enables cells to secrete a variety of growth factors, cytokines, and proteases that contribute to age-related tissue dysfunction as well as to tumour formation, and thus create a local environment that is toxic to neighbouring normal cells.

There is accordingly a need for agents that are capable of detecting senescent cells and for depleting senescent cells, thereby to diagnose and treat conditions associated with cell senescence.

Summary of the Invention

It has now been discovered that senescent cells manifest a surface marker that can be targeted for therapeutic and diagnostic purposes. The marker is related to a surface protein that is native to the cell in its healthy state. Particularly, it has been found that certain native surface proteins adopt a misfolded conformation when the host cell is senescent. Accordingly, the present invention exploits, as target, a misfolded form of a senescent cell surface protein. The misfolded surface protein is unique in conformational terms relative to the native protein, and allows for the production and use of binding agents, such as antibodies, that bind selectively to the misfolded form of the protein.

In one of its aspects, the present invention provides a method for detecting senescent cells, in which a binding agent that binds selectively to a misfolded surface protein, i.e., the marker protein, is combined with a sample containing cells to be examined, and determining whether the cells are bound by the binding agent to form a complex, the presence of such complex indicating that the sample contains senescent cells.

The assay method can be performed using techniques such as flow cytometry, ELISA, Western blot and the like. The assay can be practised *in vitro*, and *in vivo* by way of imaging to reveal senescent cells endogenous to a subject.

In another of its aspects, the present invention provides a method useful to deplete senescent cells endogenous to a subject and/or to treat an age-related disease in a subject in need thereof, comprising administering to the subject a binding agent that is selectively toxic to senescent cells in an amount effective to reduce the number of such cells, wherein the binding agent binds selectively to a senescent cell surface protein having a misfolded conformation.

In embodiments, the binding agent is an antibody or fragment thereof that binds selectively to the marker protein. Suitable binding agents are agents that bind the marker protein at least two fold more efficiently than its native protein counterpart. Particularly suitable binding agents are antibodies, and fragments thereof that bind the marker protein with comparable efficiency. Also suitable are antibody conjugates in which the antibody or binding fragment is conjugated with a toxin useful to kill the targeted senescent cells.

In a further aspect, the present invention provides a method useful to deplete senescent cells endogenous to a subject in need thereof and/or to treat an age-related disease in a subject in need thereof, comprising administering to the subject an immunogen, such as in the form of a vaccine, that elicits an antibody that

is selectively toxic to senescent cells, wherein the antibody binds selectively to a senescent cell surface protein having a misfolded conformation.

In one specific embodiment, the marker protein is a misfolded form of prion protein, PrP. In other embodiments, the marker protein is a misfolded form of a cell surface protein such as Fas receptor (FasR). In further embodiments, the marker protein is a misfolded form of CD38, Notch-1, CD44, CD59, Fas ligand, TNF receptor, or EGF receptor.

In embodiments, the present method exploits particularly an antibody that binds selectively to a misfolded form of human FasR, as well as misfolded FasR-binding antibody fragments and conjugates thereof, and pharmaceutical compositions and medical uses thereof. In addition, there is provided a method for producing the antibody, and immunogens/vaccines that elicit endogenous production of the antibody.

These and other aspects of the invention are now described in greater detail with reference to the accompanying drawings in which:

Brief Description of the Figures

Figure 1 shows gating of live cells by flow cytometry based on forward scatter (FSC), side scatter (SSC), and viability dye exclusion (PerCP-Cy5.5).

Figure 2 shows the binding of isotype control antibodies (mIgG and rIgG, mouse IgG and rabbit IgG, respectively), secondary antibody alone (2°Ab), the pan-PrP specific antibody 6D11, and the misfolding specific anti-PrP antibody AMF-1c-20 (DSE) to untreated and mitomycin c-treated senescent HUVEC cells. Analysis is by flow cytometry.

Figure 3 shows gating of live cells by flow cytometry based on forward scatter (FSC), side scatter (SSC), and 7AAD viability dye exclusion (PerCP-Cy5.5).

Figure 4 shows binding isotype control antibodies (mIgG and rIgG, mouse IgG and rabbit IgG, respectively), secondary antibody alone (2°anti-m and 2°anti-r), the pan-PrP specific antibody 6D11, and the misfolding specific anti-Fas antibodies AMF-3a-118 and AMF-3d-19 to untreated (A) and mitocmycin c-treated senescent (B) HUVEC cells. Analysis is by flow cytometry.

Detailed Description of the Invention

The invention is based on the discovery of a senescent cell marker that can be targeted for diagnostic and medical intervention. The marker is a cell surface protein that has adopted a misfolded conformation on cells that are senescent. The marker is thus related to a protein found in a natively folded state on the

surface of a normal, healthy cell. When the cell has entered a senescent state, however, the marker protein is misfolded, and presents regions or domains that are not found or accessible in the natively folded protein. These unique regions or domains serve as epitopes for the raising of antibodies and selection of other binding agents that, in turn, bind uniquely and selectively to the misfolded protein.

The present methods are applied diagnostically to detect senescent cells present in any given sample, including biological fluids or tissues obtained from a subject, or to detect senescent cells in situ, i.e., senescent cells that are endogenous to a subject.

The present methods are also applied therapeutically to deplete senescent cells endogenous to a subject in need of treatment. Senescent cell "depletion" refers simply to a reduction in the number of viable senescent cells. Without wishing to be bound by theory, the result of eliminating or depleting senescent cells is to reduce the effect of these cells on their neighbouring environment, such as to reduce the so-called senescence-associated secretory phenotype (SASP) effect through which senescent cells create a toxic local environment that affects the health of neighbouring cells and promotes cell aging and associated medical conditions, including tumourigenesis.

The invention is thus based on the principle that misfolded proteins are present on the surface of senescent cells, and that senescent cells can therefore be identified and their deleterious effects controlled, reduced or eradicated by targeting the misfolded form of that surface protein. In one approach, senescent cells are screened to identify surface proteins that have adopted a conformation different from normal cells that present the protein in natively folded state. Once identified, the surface protein can be examined *in silico* using established algorithms to identify misfolding "hot spots" within its sequence. One such algorithm is described by Cashman et al in WO 2010/040209, incorporated herein by reference. As described in that publication, a wide variety of cell surface proteins have been examined and epitopes that are uniquely presented when that protein misfolds are identified. These epitopes are particularly useful targets for the methods of the present invention.

Senescent cell surface protein epitopes that are presented "uniquely" by the misfolded form of a protein, relative to the natively folded form of that protein, thus provide a basis for distinguishing between the two forms of the protein, and allow the misfolded protein to be targeted while substantially removing the natively folded protein as a complicating factor in therapeutic and diagnostic applications. In principle, an epitope is unique to a misfolded protein if an antibody or other ligand that binds that epitope shows significantly reduced binding to the natively folded form of that protein. In an embodiment, the binding difference is effective to yield a discernible test result or therapeutic result. The misfolded form of a protein is characterized, in the present context, as a form that presents an epitope not found in the natively

folded protein. This results from the protein adopting a different conformation, owing for example to different environmental conditions (for example, oxidation of cysteine), defects in protein folding surveillance (for example, ineffective chaperone function), aberrant post-translational modifications (for example, defective maturation of glycosylation), abnormal interaction with another protein (for example, PrPC/PrPSc interaction), or from genetic mutation. As a result of misfolding, the protein can adopt an unusual and undesirable state, with a different immunogenicity relative to its normal state.

Accordingly, an aspect of the invention includes a method to deplete senescent cells endogenous to a subject, comprising administering to the subject a binding agent that is selectively toxic to senescent cells in an amount effective to reduce the number of such cells, wherein the binding agent binds selectively to a senescent cell surface protein having a misfolded conformation.

The term "selectively toxic to senescent cells" means that the binding agent is at least 2 times more toxic to a senescent cell expressing the misfolded protein, compared to a cell such as a normal not expressing the misfolded protein , such as 3 times greater, 5 times greater or at least one order of magnitude greater (e.g., at least 2, 3, 4 or 5 orders of magnitude greater). For instance, the cell toxicity of an antibody that binds misfolded PrP on a senescent cell is preferably at least twice its toxicity for natively folded PrP on normal tissue.

Another aspect includes a method to treat an age-related disease in a subject in need thereof, comprising administering to the subject a binding agent that binds selectively to a senescent cell surface protein having a misfolded conformation.

The binding agent binds selectively to an epitope exposed in a misfolded conformation of the cell surface protein present on the senescent cell. The term "epitope" refers a region of a protein that is recognized by a B cell or T-cell receptor, or an antibody or a binding fragment thereof and is represented herein by linear peptides having amino acid sequences that are found in the parent protein in which the epitope resides. The epitope will usually comprise at least 3 contiguous amino acid residues, representing the minimal domain necessary for antibody binding. However, the epitope can include a larger number of contiguous residues, such as 5 residues, 7 residues which typically is the length required for minimal immunogenicity within common antibody production hosts, 10 residues, 15 residues, 20 residues or more. The criticality lies in providing an epitope that harbours an antibody-binding domain. Thus, and with reference to Tables 1, 2 and 3, *infra*, it is to be appreciated that the peptides specifically listed, in addition to serving per se as epitopes, may further comprise additional amino acid residues at one or both flanks thereof, particularly for those peptides that consist of fewer than 7 residues. The additional residues can be those normally associated with the peptide in the context of the parent protein. Conversely, it should be

appreciated that truncated forms of the peptides can also serve as epitopes, especially when the peptide consists of more than 7 residues. The extent of truncation can vary depending on the actual length of a given peptide. Any contiguous 7 residues within a listed peptide can be expected to be immunogenic per se, and any 3, 4, 5, 6, or 7 contiguous residues or more can be expected to be useful as an epitope to which antibodies can bind.

The senescent cell marker can be any surface protein that is misfolded on the surface of a senescent cell, but is otherwise natively folded on a normal cell counterpart. Of course, the variety of surface proteins that are candidates as senescent cell markers are numerous. The determination that a given surface protein is a senescent cell marker can be made by comparing the conformations of that protein on a normal cell vs. a senescent cell of the same type. In embodiments, the surface protein is selected from prion protein (PrP), FasR, Fas ligand, CD44, EGF receptor, CD38, Notch-1, CD44, CD59, or TNF receptor, and the like.

Prion Protein, PrP, as Target

In embodiments of the present invention, the senescent cell marker is a misfolded form of prion protein, or PrP. In mature form, the human prion protein (hPrPC) is a 230 residue protein having the sequence set out in UniProtKB/SwissProt designation P04156. This protein is known to adopt a misfolded conformation when associated with neurodegenerative diseases including CJD, and when presented on the surface of certain cancer cells, including particularly ovarian cancer cells. Accordingly, various antibodies specific for the misfolded forms of PrP have been developed and are described in the literature. These antibodies and vaccines that produce them are useful in the present invention.

More particularly, the present invention exploits agents that bind uniquely to misfolded PrP, and particularly, in embodiments of the present invention, to an epitope comprised by any one of the following peptides:

Table 1

Protein Target (abbr) UniProtKB/SwissProt #	Residue span	Sequence	SEQ ID No.
Human prion protein (hPrP) P04156 residues 1-230	125-130	LGGYML	1
	126-132	GGYMLGS	2

	140-147	HFGSDYED	3
	143-147	SDYED	4
	148-155	RYYRENMH	5
	151-155	RENMH	6
	160-166	QVYYRPM	7
	165-174	PMDEYSNQNN	8
	166-174	MDEYSNQNN	9
	185-197	KQHTVTTTTKGEN	10
	70-80	ARDCTVNGDEP	11
	105-111	RLCDEGH	12
	136-142	NSTVCEH	13
	167-189	EEPSRSNLGWLCL	14

In a particular embodiment, the binding agent binds selectively to an epitope that comprises a YYR motif. As shown in Table 1, the YYR motif lies within PrP residues 160-166 (SEQ ID No.7) and within PrP residues 148-155 (SEQ ID No.5). These peptides and the epitopes therein are accordingly useful targets for the binding agent. It will be appreciated that variations of these specific peptides that also comprise YYR and are based also on the PrP protein sequence are included as useful targets for the binding agent. Such peptides will typically include at least 5 contiguous residues, for instance, RYYRE (SEQ ID No.15), RYYREN (SEQ ID No.16), RYYRENM (SEQ ID No.17), DRYYRENMH (SEQ ID No.18), DRYYRENM (SEQ ID No. 19), and VYYRPM (SEQ ID No.20), QVYYRP (SEQ ID No.21), QVYYR (SEQ ID No.22), QVYYRPMD (SEQ ID No.23), and the like.

In another embodiment, the binding agent is one that binds selectively to a misfolded PrP epitope that comprises a YML motif. This particular motif lies within PrP beta strand 1. As shown in Table 1 above, the YML motif is common within PrP peptides, LGGYML and GGYMLGS (SEQ ID Nos. 1 and 2, respectively). These peptides are accordingly also useful epitope targets for the binding agent. It will be appreciated that variations of these specific peptides that also comprise YML and are based also on parent protein sequence are included as useful epitope targets in embodiments of the present invention. Such peptides will typically include at least 3, 4 or more usually at least 5 contiguous residues, for instance, GGYMLG (SEQ ID NO: 25), GYMLGS (SEQ ID NO: 26), GGYML (SEQ ID NO: 27), YMLGS (SEQ ID NO: 28), GYML (SEQ ID NO: 29), YMLG (SEQ ID NO: 30) LGGYML (SEQ ID No. 31), LGGYMLG (SEQ ID No. 32) and YML (SEQ ID NO: 33).

In a further embodiment, the binding agent binds selectively to a misfolded PrP epitope that comprises a MDEYSNQNN motif. This particular motif, located between β-sheet 2 and α-helix 1, is known also as the rigid loop, and lies within PrP residues 167-174, as shown in Table 1. It will be appreciated that variations of these specific peptides that also comprise at least about 5 contiguous residues within the rigid loop sequence and are based also on parent protein sequence are included as useful epitope targets in embodiments of the present invention. Such peptides will typically include at least 5 contiguous residues, for instance, PMDEYSNQNN (SEQ ID No.34), DEYSNQNN (SEQ ID No.35), and MDEYSNQ (SEQ ID No.36).

Agents that bind to these epitopes will bind selectively to misfolded PrP, relative to natively folded PrP, and will thus bind selectively to senescent cells in which surface PrP is misfolded, relative to normal cells.

Agents that bind to these epitopes can be identified using routine screening methods in which a panel of putative ligands is combined with a peptide presenting the epitope, and binding events are then analyzed to identify peptide binding partners. Ligands that also bind to PrP in its wild type, natively folded state can be dismissed, so that only the ligands or binding partners that bind uniquely to the epitope targets are selected.

It will be appreciated that the binding agents useful herein can take the form of small molecules, peptides, peptidomimetics, peptide or nucleic acid aptamers, and the like. In particular embodiments, the binding agent is an antibody or an epitope-binding fragment thereof. In other embodiments, the binding agent is induced by the administration of an immunogen that elicits production within the recipient of antibodies that bind the senescent cell marker.

In embodiments, the binding agent is an antibody that binds selectively to PrP in a misfolded form presented by senescent cells. In particular embodiments, the antibody is one that binds selectively to a peptide listed in Table 1.

In more specific embodiments, the antibody is one that binds a peptide incorporating the YML motif of PrP. Particularly, the antibody is the antibody designated 1A1, as described in more detail in WO 2010/099612 incorporated herein by reference. This antibody displays selective binding to the prion protein YML motif. Its hybridoma source was deposited under terms of the Budapest Treat at the International Depositary Authority of Canada (IDAC) under accession number 260210-01.

In other specific embodiments, the antibody is one that binds a peptide incorporating the YYR motif of PrP. Particularly, the antibody is one that is raised in the manner described in WO 03/000853 (and see US 7,041,807) incorporated herein by reference, and as reproduced in Example 1 herein for convenience.

In another specific embodiment, the antibody is one that binds the rigid loop of PrP. Particularly, the antibody is the antibody designated c-120, as described in co-pending WO2013/185215 published on 2013 Dec 19, and incorporated herein by reference. This antibody is defined by heavy and light chains having the sequences set out in SEQ ID No.38 and SEQ ID No. 37, respectively.

The variable regions of the antibody have the sequences set out in SEQ ID No.39 for the light chain variable region, and in SEQ ID No.40 for the heavy chain variable region.

The sequences of the important complementarity determining regions of the c-120 antibody have the following sequences:

For the heavy chain:

CDR1	TYAMG	(SEQ ID No. 41)
CDR2	VITKSGNTYYASWAKG	(SEQ ID No. 42)
CDR3	YGIGVSYYDI	(SEQ ID No. 43)

For the light chain:

CDR1	QSSQSLYNKNWLS	(SEQ ID No. 44)
CDR2	KASTLES	(SEQ ID No. 45)
CDR3	QGEFSCSSADCTA	(SEQ ID No. 46)

It will be appreciated that the binding agent thus can be an antibody or a fragment thereof that binds selectively to the rigid loop of PrP and incorporates at least the CDR sequences provided above.

Cell surface proteins other than PrP are also misfolded when the host cell is in a state of senescence. Like PrP, the conformational alteration results in the presentation of new epitopes that are unique to the misfolded protein, relative to its native counterpart. These epitopes are useful targets for senescent cell detection and depletion. More particularly, the present inventors have applied an algorithm that predicts misfolding "hot spots" within surface proteins. That algorithm is described by Cashman et al in WO 2010/040209, and was used to identify the misfolding specific epitopes within the target surface proteins shown below.

Table 2

Protein Target (abbr) UniProtKB/SwissProt #	Residue span	Sequence	SEQ ID No.
Human CD44 P16070 residues 21-742	39-45	NGRYSIS	47
	83-87	EGHVV	48
	108-116	TSNTSNYDT	49
	149-159	NRDGTRYVQKG	50
	99-116	ANNTFVYILTSNTSNYDT	51
Human tumour necrosis factor receptor (hTNFR) P19438	21-31	IHPQNNSICCT	52
	41-51	NDCPGPGQDTD	53
	106-112	YWSENLF	54
	126-134	HLSCQEKQN	55
	139-151	CTCHAGFFLRENECV	56
Human notch protein (hNOTCH1) P46531 residues 1-2550	423-429	CEHAGKC	57
	479-487	MPGYEGVHC	58
	499-505	CLHNGRC	59
	508-512	KINEF	60
	493-517	ECASSPCLHNGRCLDKINEFQCE CP	61
Human Fas receptor P25445 residues 26-335	52-60	LHHDGQFCH	62
	70-80	ARDCTVNGDEP	63
	105-111	RLCDEGH	64
	136-142	NSTVCEH	65
	167-189	EEGSRSNLGWLCL	66
Human epidermal growth factor receptor (hEGFR) P00533 residues 25-1210	11-25	SNKLTQLFTFEDHFL	67
	46-56	VQRNYDLSFLK	68
	83-95	IRGNMYYENSYAL	69
	97-107	VLSNYDANKTG	70
	143-155	IVSSDFLSNMSMD	71
	148-169	FLSNMSMDFQNHLGS	72
	176-181	WGAGEE	73

	241-259	PPLMLYNPTTYQMDVNPE	74
	257-266	PEGKYSFGAT	75
	273-282	RNYVVTDHGS	76
	288-301	GADSYEMEEDGVRK	77
	314-328	NGIGIGEFKDSLSIN	78
	328-337	ATNIKHFKN	79
	348-363	LPVAFRGDSFTHTPPL	80
	465-474	KIISNRGENS	81
Human CD38 P28907 1-300	70-84	YTEIHPEMRHVDCQS	82
	161-169	GEFATSKIN	83
	176-182	WRKDCSN	84
	210-220	GSRSKIFDKDS	85
	243-253	IHGGREDSRDL	86

It has been determined further that the cell surface protein human FasR (Fas receptor) is also misfolded when present on senescent cells. Fas receptor (FasR) is known also as human tumour necrosis factor superfamily member 6 receptor (hTNFRSM6), and as CD95, and is implicated in cancer. It is a death receptor on the surface of cells that leads to caspase-mediated programmed cell death (apoptosis). More particularly, the present invention provides and exploits agents that bind uniquely to misfolded FasR, and particularly, in embodiments of the present invention, to an epitope comprised by any one of the peptides shown in Table 3:

Table 3

Human FasR (hTNFRSM6) P25445 residues 26-335	52-60	LHHDGQFCH	62
	70-80	ARDCTVNGDEP	63
	105-111	RLCDEGH	64
	136-142	NSTVCEH	65
	167-189	EEPSRSNLGWLCL	66

In a particular embodiment, the binding agent binds selectively to a FasR epitope that comprises an LHHDGQFCH sequence (SEQ ID No.62). In an alternative embodiment, the binding agent binds selectively to a FasR epitope that comprises an NSTVCEH sequence (SEQ ID No.65).

Also provided herein as binding agents that bind selectively to misfolded FasR, are antibodies that bind to one of the two peptide sequences just recited. In particular, and in one of its aspects, the present invention provides an antibody designated AMF 3a-118, the antibody having been raised against the LHHDGQFCH in the manner exemplified herein. The heavy chain of the 3a-118 antibody has SEQ ID No. 89. The light chain of the 3a-118 antibody has SEQ ID No. 91. The heavy chain variable region of the 3a-118 antibody has SEQ ID No. 90. The light chain variable region of the 3a-118 antibody has SEQ ID No. 92.The misfolded FasR binding site presented by this antibody comprises the following CDRs:

For the heavy chain

CDR1 DSRVS (SEQ ID No. 93)

CDR2 IVGIGWNIYHANWAKG (SEQ ID No. 94)

CDR3 GLGGGTVI (SEQ ID No. 95)

For the light chain

CDR1 QSSESVYKNNYLS (SEQ ID No. 96)

CDR2 EASKLAS (SEQ ID No. 97)

CDR3 LGEFSCYSGDCGT (SEQ ID No. 98)

In addition, the present invention provides an antibody designated AMF 3d-19, the antibody having been raised against the NSTVCEH sequence in the manner exemplified herein. The heavy chain of the 3d-19 antibody has SEQ ID No. 103. The light chain of the 3d-19 antibody has SEQ ID No. 105. The heavy chain variable region of the 3d-19 antibody has SEQ ID No. 104. The light chain variable region of the 3d-19 antibody has SEQ ID No. 106. The misfolded FasR binding site presented by this antibody comprises the following CDRs:

For the heavy chain

CDR1 RNAIN (SEQ ID No. 107)

CDR2 IIGSSGVTYYASWAKG (SEQ ID No. 108)

CDR3 NLYTGGSNDNL (SEQ ID No. 109)

For the light chain

CDR1 QASKSVYNNVQLS (SEQ ID No. 110)

CDR2 YASTLAS (SEQ ID No. 111)

CDR3 AGGYSSSSDNA (SEQ ID No. 112)

It will thus be appreciated that the binding agent useful in the present methods can be any antibody or a fragment thereof that binds selectively to a protein that is misfolded on the surface of a senescent cell, by binding to a particular epitope that is unique to that misfolded form relative to the natively folded protein.

The antibodies that bind selectively to the misfolding specific epitopes may be either polyclonal or monoclonal, of the IgG or IgM class, and may be derived from any mammal, particularly goats, rabbits or mice, or by recombinant methods. More generally, it will be appreciated that antibodies useful in the present invention include the various intact forms including polyclonal antibodies, monoclonal antibodies, and recombinant antibodies including chimeric antibodies, humanized antibodies as well as fully human antibodies and bispecific or multispecific antibodies.

In an embodiment, the antibody comprises a human constant region.

The chimeric antibodies comprise a portion of the heavy and/or light chain that is homologous with 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 homologous with corresponding sequences derived from another species or belonging to a different antibody class. Humanized antibodies are chimeric antibodies that comprise minimal sequence derived from non-human antibody, usually incorporating CDRs from a non-human antibody into a human antibody framework, which may further be altered to incorporate non-human residues that restore and enhance antigen binding. The "fully" human antibodies can be produced in a non-human host using various techniques that are now established, including through the use of phage display libraries, and particularly by introducing human immunoglobulin loci into transgenic animals such as mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibodies are produced which closely resemble that seen in humans in most respects, including gene rearrangement, assembly

and antibody repertoire.

Using phage display antibodies are displayed on the surface of phage by for example fusing the coding sequence of antibody variable regions to the phage minor coat protein pIII. Antibodies can be selected using the phage displayed antibody libraries (including synthetic antibody libraries wherein synthetic diversity is introduced at solvent-exposed positions within the heavy chain complementarity-determining regions) by a series of cycles of selection on antigen. Antibody genes can be cloned simultaneously with selection and further engineered for example by increasing their affinity or modulating their specificity or their effector function (by recloning into a full-length immunoglobulin scaffold e.g. making a recombinant human antibody).

In an embodiment, the antibody is a recombinant or synthetic antibody, for example the human antibody is a recombinant or synthetic human antibody.

The antibodies may be of any useful class, including IgA, IgD, IgE, IgG and IgM, and isotypes including IgG1, IgG2, IgG3, and IgG4. The constant region (Fc) of the antibodies can also be engineered or conjugated to provide altered effector function, thereby to enhance antibody dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) activity.

For therapeutic use, in embodiments, the antibody desirably is itself toxic to the senescent cells presenting the target epitope. To this end, the antibody desirably incorporates an Fc region having effector function, such as ADCC activity and/or CDC activity. In the alternative, and in accordance with other embodiments, the antibody or binding fragment is conjugated with a cytotoxin that is toxic to the senescent cells targeted by the antibody or binding fragment.

The antibodies otherwise can have all of the attributes common to intact antibodies. In embodiments, the present antibodies are of the IgG1 isotype, but they may also be IgG2 or IgG4. Moreover, the isotype of the antibody, as dictated by the constant region, can be manipulated to alter or eliminate the effector function of the resulting antibody. That is, the constant region of the present antibodies is either wild type human antibody constant region, or a variant thereof that incorporates amino acid modifications, i.e., amino acid additions, substitutions or deletions that alter the effector function of the constant region, such as to enhance serum half-life, reduce or enhance complement fixation, reduce or enhance antigen-dependent cellular cytotoxicity and improve antibody stability. The number of amino acid modifications in the constant region is usually not more than 20, such as 1-10 e.g., 1-5 modifications, including conservative amino acid substitutions.

In embodiments, the half-life of the antibody is improved by incorporating one or more amino acid modifications, usually in the form of amino acid substitutions, for instance at residue 252, e.g., to introduce Thr, at residue 254, e.g., to introduce Ser, and/or at residue 256 e.g., to introduce Phe. Still other modifications can be made to improve half-life, such as by altering the CH1 or CL region to introduce a salvage receptor motif, such as that found in the two loops of a CH2 domain of an Fc region of an IgG. Such alterations are described for instance in US 5869046 and US 6121022.

Altered C1q binding, or reduced complement dependent cytotoxicity, can be introduced by altering constant region amino acids at locations 329, 331 and 322, as described in US 6194551. The ability of the antibody to fix complement can further be altered by introducing substitutions at positions 231 and 239 of the constant region, as described in WO94/029351.

Framework modifications can also be made to reduce immunogenicity of the antibody or to reduce or remove T cell epitopes that reside therein, as described for instance by Carr et al in US2003/0153043.

Antibodies can also be altered in the variable region to eliminate one or more glycosylation sites, and/or to improve physical stability of the antibody. For example, in one embodiment, the physical stability of the antibody is improved by substituting the serine at position 228 of the variable region with a proline residue (i.e., the antibody has a variable region comprising a S228P mutation). The S228P alteration significantly stabilizes the antibody structure against the formation of intrachain disulfide bonds. In another embodiment, the variable region is altered to eliminate one or more glycosylation sites resident in the variable region. More particularly, it is desirable in the sequence of the present antibodies to eliminate sites prone to glycosylation. This is achieved by altering the occurrence of one or more N-X-(S/T) sequences that occur in the parent variable region (where X is any amino acid residue), particularly by substituting the N residue and/or the S or T residue.

Antibodies can be engineered to include a variety of constant regions. In one embodiment, the antibody comprises a constant region the sequence of which corresponds to the constant region of an antibody of human origin, such as a human IgG1 constant region. In a particular embodiment, the constant region is inert for effector function (e.g., essentially devoid of effector function). In a specific embodiment the constant region is a human IgG4 constant region.

In yet another embodiment, the Fc region is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase the affinity of the antibody for an Fcγ receptor by modifying one or more amino acids at the following positions: 238, 239, 248, 249, 252, 254, 255, 256, 258, 265, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294,

295, 296, 298, 301, 303, 305, 307, 309, 312, 315, 320, 322, 324, 326, 327, 329, 330, 331, 333, 334, 335, 337, 338, 340, 360, 373, 376, 378, 382, 388, 389, 398, 414, 416, 419, 430, 434, 435, 437, 438 or 439. This approach is described further in PCT Publication WO 00/42072. Moreover, the binding sites on human IgG1 for FcγR1, FcγRII, FcγRIII and FcRn have been mapped and variants with improved binding have been described (see Shields et al. (2001) J. Biol. Chem. 276:6591-6604). Specific mutations at positions 256, 290, 298, 333, 334 and 339 were shown to improve binding to FcγRIII. Additionally, the following combination mutants were shown to improve FcγRIII binding: T256A/S298A, S298A/E333A, S298A/K224A and S298A/E333A/K334A.

In addition, the antibody can be pegylated, for example, to increase the biological (e.g., serum) half-life of the antibody. To pegylate an antibody, the antibody, or fragment thereof, typically is reacted with polyethylene glycol (PEG), such as a reactive ester or aldehyde derivative of PEG, under conditions in which one or more PEG groups become attached to the antibody or antibody fragment. Preferably, the pegylation is carried out via an acylation reaction or an alkylation reaction with a reactive PEG molecule (or an analogous reactive water-soluble polymer). As used herein, the term "polyethylene glycol" is intended to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono (C1-C10) alkoxy- or aryloxy-polyethylene glycol or polyethylene glycol-maleimide. In certain embodiments, the antibody to be pegylated is an aglycosylated antibody. Methods for pegylating proteins are known in the art and can be applied to the antibodies of the invention. See, e.g., EP 154316 and EP 401384.

The antibodies and binding fragments are useful both for diagnostic purposes, including *in vivo* imaging to identify endogenous sites of senescent cells *in vivo*, and for sample testing to detect senescent cells *in vitro*. The antibodies and binding fragments are also useful for therapeutic purposes to treat diseases in which senescent cells are implicated.

"Selective-binding" agents are agents that bind the target epitope, and bind proteins that present the target epitope in a solvent-accessible orientation, with an affinity that is at least 2 times greater than the affinity with which they bind a different, unrelated epitope, such as 3 times greater, 5 times greater or at least one order of magnitude greater (e.g., at least 2, 3, 4 or 5 orders of magnitude greater For instance, the binding affinity of an antibody that binds misfolded PrP on a senescent cell is preferably at least twice its binding affinity for natively folded PrP on normal tissue. Relative binding affinities can be determined, and the antibody so selected, on the basis of assays and techniques that generally are well established in the art for this purpose.

In an embodiment, the antibody affinity has an EC50 which is at least 10^{-6} M, at least 10^{-7} M, at least 10^{-8}

M, or at least 10^{-9} M.

All antibodies were then tested by ELISA for binding to denatured full-length recombinant PrP. One antibody exhibited a titer for denatured PrP that was similar to the anti-peptide affinities, in the 10^{-10} M range. Thus, the preferred antibody exhibits preferably an EC50 by this test that is at least better than 10^{-9}M.

Antibodies that bind selectively to the epitope unique to a misfolded form of a protein can be produced by techniques including immunization, or by alternative approaches such as by the application of phage display and other systems that use high throughput to identify complementarity determining regions (CDR) or other sequences that bind to the target epitope. It is not essential that the resulting antibody has been first raised *in vivo*. More particularly, to produce suitable antibodies, amino acid sequences that constitute epitopes can be useful *per se* to raise antibodies that bind specifically to them, provided they are endowed *per se* with the immunogenicity required to raise antibody in the selected antibody production host. For those epitopes that lack such immunogenicity, it is desirable to provide an immunogen that contains the epitope sequence.

As used herein, "immunogen" refers to an immunogenic form of a peptide or other molecule that comprises the epitope, and is represented by the peptide itself when immunogenic per se, or is represented by the peptide in combination with an immunogenicity-enhancing agent. Any of the established agents can be used for this purpose. These agents typically include carrier proteins that can be coupled to the epitope either directly, such as through an amide bond, or indirectly through a chemical linker such as carbodiimide, a cysteine, or any peptide spacer sequence such as a glycine or glycine-serine sequence including Gly4-S. For example, an isolated peptide comprising a given epitope can be conjugated to MAP antigen, OVA antigen, or keyhole limpet hemocyanin (KLH). Its large size makes it very immunogenic, and the large number of lysine residues available for conjugation makes KLH very useful to attach to a polypeptide. The immunogen may further comprise a linker effective to couple the peptide tandemly to another copy of the same or a different peptide corresponding to the same or a different epitope. In another embodiment, the peptides may comprise additional amino acids that enhance the immunogenicity or solubility of the peptide. In one embodiment, the additional amino acids number from 1 to about 10, preferably 1 to 8, more preferably 1 to 5. Importantly the additional residues do not materially affect the conformation of the peptide.

Thus, for antibody production, epitopes that are not themselves immunogenic and do not constitute an immunogen can be rendered so, and provided as an immunogen, by incorporating immune enhancing agents that are either conjugated therewith or coupled covalently.

A composition comprising the immunogen can be prepared for purposes of producing antibodies in a selected host by combining the immunogen with an appropriate vehicle. Such vehicles include Freund's complete adjuvant or other adjuvant or a suitable saline or phosphate buffered saline solution (0.05 – 1.0%).

Antibodies are then prepared to react against these epitopes when they are in an unstructured state. As noted, each peptide may be conjugated to a carrier protein like KLH to form an immunogen that is injected, optionally in combination with an adjuvant such as Freund's complete adjuvant, into a mammalian production host like a mouse, rat, rabbit, sheep or goat to provoke an immune response that generates antibodies against the peptide. Standard immunization protocols can be used, and the antibodies can be recovered from blood by enrichment against the immunizing agent, as exemplified herein.

The antibodies useful herein are desirably "isolated" antibodies, which refers to an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds misfolded PrP is substantially free of antibodies that specifically bind antigens other than PrP proteins). An isolated antibody that specifically binds a misfolded human PrP protein may, however, have cross-reactivity to other antigens, such as misfolded PrP proteins from other species. Moreover, an isolated antibody can be substantially free of other cellular material and/or chemicals. An isolated antibody also can be substantially free of other proteins of human origin. In embodiments, the isolated antibody is an exogenous antibody as distinct from an antibody endogenous to an intended recipient.

Thus, in embodiments, the antibody is an intact antibody comprising features common to all natural antibodies, e.g., a heavy chain and a light chain, each chain having a constant region and a variable region, each variable region comprising framework regions (FRs) and complementarity determining regions (CDRs). In the alternative, the antibody is provided as a target-binding fragment, such as a monovalent fragment, Fab, or a bivalent antibody fragment comprising both "arms" of an intact antibody, joined through a linker that can be represented by the hinge region of the antibody or any equivalent. Such fragments include F(ab)2 fragments and any other fragment that retains preference for binding to misfolded PrP. In particular embodiments, the antibody fragment is a F(ab')2 fragment, generated for instance by papain-based digestion of the parent antibody using standard procedures for digestion and subsequent fragment isolation. In the alternative, the fragment can be a so-called single chain Fv (scFv), consisting of the variable light and variable heavy antibody domains joined by an amino acid linker, or a bivalent form of a so-called diabody prepared using a 5 amino acid linker such as SGGGG (SEQ ID NO:

117) between the light and heavy chain variable domains and a C-terminal cysteine modification to GGC to give a final diabody product as VL-SGGG-VH-GGC. Still other bivalent fragments can be prepared by coupling the light and heavy chain variable domains through thioether linkages such as bis-maleimidomethyl ether (BMME), N,N'-p-phenylene dimaleimide (PDM and N,N'-bismaleimidohexane BMH), to stabilize the F(ab')2 fragments.

Of course, for antibodies having known protein or gene sequences, the antibody can be produced suitably by recombinant DNA means. For production, there is provided a DNA molecule that encodes the heavy chain of the present antibody, and a DNA molecule that encodes the light chain thereof. The DNA further encodes any suitable signal peptide suitable for expression of a secretable chain precursor that enables proper externalization with folding and disulfide formation to elaborate the desired antibody as a secreted, dimerized and processed protein.

To this end, the present invention provides, in one aspect, polynucleotides that encode the heavy and light chains of the FasR antibodies herein described. In one embodiment, there is provided a polynucleotide comprising a sequence that encodes the light chain variable region of the FasR antibody AMF 3a-118, as set out in SEQ ID No.102. Also provided, in another embodiment, is a polynucleotide comprising a sequence that encodes the heavy chain variable region of the FasR antibody AMF 3a-118, as set out in SEQ ID No.100.

In more specific embodiments, the present invention provides a polynucleotide that encodes the entire light chain (SEQ ID No. 101) and a polynucleotide that encodes the entire heavy chain (SEQ ID No. 99) of misfolded FasR antibody AMF 3a-118 antibody.

The present invention also provides, in another aspect, polynucleotides that encode the light chain variable region of the FasR antibody AMF 3d-19, as set out in SEQ ID No. 116, and a polynucleotide comprising a sequence that encodes the heavy chain variable region of the FasR antibody AMF 3d-19, as set out in SEQ ID No. 114.

In more specific embodiments, the present invention provides a polynucleotide that encodes the entire light chain (SEQ ID No. 115) and a polynucleotide that encodes the entire heavy chain (SEQ ID No. 113) of the misfolded FasR antibody AMF 3d-19

It will be appreciated that polynucleotide equivalents also can be used, in which synonymous codons are replaced within the sequences provided, to produce the present antibodies. In an embodiment, the nucleic acid is a cDNA. In another embodiment, the nucleic acid is a codon optimized cDNA.

In embodiments, there are also provided vectors that comprise polynucleotides that encode the heavy chain or the variable region thereof and that encode the light chain or the variable region thereof. To express the antibodies, the polynucleotides are incorporated operably within expression vectors, i .e. operatively linked to transcriptional and translational control sequences. Expression vectors include plasmids, retroviruses, cosmids, and the like. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy gene can be inserted into separate vectors. In a preferred embodiment, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (e.g., ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present).

A convenient vector is one that encodes a functionally complete human CH or CL immunoglobulin sequence, with appropriate restriction sites engineered so that any VH or VL sequence can be easily inserted and expressed, as described above. In such vectors, splicing usually occurs between the splice donor site in the inserted J region, and the splice acceptor site preceding the human C region, and also at the splice regions that occur within the human CH exons. Polyadenylation and transcription termination occur at native chromosomal sites downstream of the coding regions. The recombinant expression vector can also encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene may be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (i.e., a signal peptide from a non-immunoglobulin protein).

Polynucleotides encoding the heavy chain and/or the light chain, and vectors comprising these can be used for transformation of a suitable mammalian host cell. Methods for introduction of heterologous polynucleotides into mammalian calls include dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, biolistic injection and direct microinjection of the DNA into nuclei. In addition, polynucleotides may be introduced into mammalian cells by viral vectors.

Mammalian cell lines useful as hosts for expression of the antibody-encoding polynucleotides include many immortalized cell lines available from the American Type Culture Collection (ATCC). These include, inter alia, Chinese hamster ovary (CHO) cells, NSO, SP2 cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., HepG2), A549 cells, 3T3 cells, and a number of other cell lines. In a specific embodiment, the polynucleotides are expressed in a HEK293 host. Mammalian host cells include human, mouse, rat, dog, monkey, pig, goat,

bovine, horse, and hamster cells. Cell lines of particular preference are selected through determining which cell lines have high expression levels. Other cell lines that may be used are insect cell lines, such as S19 cells, amphibian cells, bacterial cells, plant cells and fungal cells. When recombinant expression vectors encoding the heavy chain or antigen-binding portion thereof are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

The antibodies of the invention can be obtained as human monoclonal antibodies. Such human monoclonal antibodies can be generated using transgenic or transchromosomic mice carrying parts of the human immune system rather than the mouse system. These transgenic and transchromosomic mice include mice referred to herein as the HuMAb Mouse® and KM Mouse®, respectively, and are collectively referred to herein as "human Ig mice."

The HuMAb Mouse® (Medarex®, Inc.) contains human immunoglobulin gene miniloci that encode unrearranged human heavy (μ and γ) and κ light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous μ and κ chain loci (see e.g., Lonberg et al. (1994) Nature 368(6474): 856-859). Accordingly, the mice exhibit reduced expression of mouse IgM or κ, and in response to immunization, the introduced human heavy and light chain transgenes undergo class switching and somatic mutation to generate high affinity human IgGκ monoclonal antibodies (Lonberg et al. (1994), supra; reviewed in Lonberg (1994) Handbook of Experimental Pharmacology 113:49-101; Lonberg, N. and Huszar, D. (1995) Intern. Rev. Immunol. 13: 65-93, and Harding and Lonberg (1995) Ann. N.Y. Acad. Sci. 764:536-546). Preparation and use of the HuMAb Mouse®, and the genomic modifications carried by such mice, is further described in Taylor et al. (1992) Nucleic Acids Research 20:6287-6295; Chen et al. (1993) International Immunology 5: 647-656; Tuaillon et al. (1993) Proc. Natl. Acad. Sci. USA 90:3720-3724; Choi et al. (1993) Nature Genetics 4:117-123; Chen et al. (1993) EMBO J. 12: 821-830; Tuaillon et al. (1994) J. Immunol. 152:2912-2920; Taylor et al. (1994) International Immunology 6: 579-591; and Fishwild et al. (1996) Nature Biotechnology 14: 845-851, the contents of all of which are hereby specifically incorporated by reference in their entirety. See further, U.S. Patent Nos. 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,877,397; 5,661,016; 5,814,318; 5,874,299; 5,770,429; and 5,545,807; PCT Publication Nos. WO 92/03918; WO 93/12227; WO 94/25585; WO 97/13852; WO 98/24884; WO 99/45962 and WO 01/14424, the contents of which are incorporated herein by reference in their entirety.

In another embodiment, the human antibodies are raised using a mouse that carries human immunoglobulin sequences on transgenes and transchomosomes, such as a mouse that carries a human heavy chain transgene and a human light chain transchromosome. This mouse is referred to herein as a "KM mouse®," and is described in detail in PCT Publication WO 02/43478. A modified form of this mouse, which further comprises a homozygous disruption of the endogenous FcγRIIB receptor gene, is also described in PCT Publication WO 02/43478 and referred to herein as a "KM/FCGR2D mouse®." In addition, mice with either the HCo7 or HCo12 heavy chain transgenes or both can be used.

Additional transgenic animal embodiments include the Xenomouse (Abgenix, Inc., U.S. Patent Nos. 5,939,598; 6,075,181; 6,114,598; 6,150,584 and 6,162,963). Further embodiments include "TC mice" (Tomizuka et al. (2000) Proc. Natl. Acad. Sci. USA 97:722-727) and cows carrying human heavy and light chain transchromosomes (Kuroiwa et al. (2002) Nature Biotechnology 20:889-894; PCT Publication WO 02/092812). The contents of these patents and publications are specifically incorporated herein by reference in their entirety.

Human monoclonal antibodies also can be prepared using SCID mice into which human immune cells have been reconstituted such that a human antibody response can be generated upon immunization. See, e.g., U.S. Patent Nos. 5,476,996 and 5,698,767, the contents of which are incorporated herein by reference in their entirety.

Antibodies of the invention also can be produced in a host cell transfectoma using, for example, a combination of recombinant DNA techniques and gene transfection methods as is well known in the art (e.g., Morrison, S. (1985) Science 229:1202). In one embodiment, DNA encoding partial or full-length light and heavy chains obtained by standard molecular biology techniques is inserted into one or more expression vectors such that the genes are operatively linked to transcriptional and translational regulatory sequences. In this context, the term "operatively linked" is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene.

The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, e.g., in Goeddel (Gene Expression Technology. Methods in Enzymology 185, Academic Press, San Diego, CA (1990)). Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV), Simian Virus 40 (SV40), adenovirus, (e.g., the adenovirus major late promoter (AdMLP) and polyoma.

Alternatively, nonviral regulatory sequences can be used, such as the ubiquitin promoter or ϐ-globin promoter. Still further, regulatory elements composed of sequences from different sources, such as the SRα promoter system, which contains sequences from the SV40 early promoter and the long terminal repeat of human T cell leukemia virus type 1 (Takebe et al. (1988) Mol. Cell. Biol. 8:466-472). The expression vector and expression control sequences are chosen to be compatible with the expression host cell used.

The antibody light chain gene and the antibody heavy chain gene can be inserted into the same or separate expression vectors. In preferred embodiments, the variable regions are used to create full-length antibody genes of any antibody isotype by inserting them into expression vectors already encoding heavy chain constant and light chain constant regions of the desired isotype such that the VH segment is operatively linked to the CH segment(s) within the vector and the VL segment is operatively linked to the CL segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (i.e., a signal peptide from a non-immunoglobulin protein).

In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the invention can carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see, e.g., U.S. Pat. Nos. 4,399,216; 4,634,665 and 5,179,017). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr-host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is theoretically possible to express the antibodies of the invention in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells, and most preferably mammalian host cells, is the most preferred because mammalian cells are more

likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody.

Preferred mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin, (1980) Proc. Natl. Acad. Sci. USA 77:4216-4220, used with a DHFR selectable marker, e.g., as described in R. J. Kaufman and P. A. Sharp (1982) J. Mol. Biol. 159:601-621), NSO myeloma cells, COS cells, HEK293 cells and SP2 cells. In particular, for use with NSO myeloma cells, another preferred expression system is the GS gene expression system disclosed in WO 87/04462, WO 89/01036 and EP 338,841. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

Thus, in another aspect, there is provided a cellular host that incorporates expressibly therein a polynucleotide that encodes the light chain, or the light chain variable region, of the misfolded human FasR antibody designated 3a-118, the polynucleotides having SEQ ID No. 101 (entire light chain) or SEQ ID No,102, respectively. In other embodiments, there is provided a cellular host that incorporates expressibly therein a polynucleotide that encodes the heavy chain, or the heavy chain variable region, of the misfolded human FasR antibody designated 3a-118, having SEQ ID No. 99 (entire heavy chain) or SEQ ID No. 100 (heavy chain variable region). In a further embodiment, there is provided a cellular host that incorporates expressibly therein both the heavy and light chain-encoding polynucleotides just recited, in either fully length form or in the form of variable region-encoding polynucleotides. In a still further embodiment, there comprises the step of culturing the transfected cellular host, thereby to produce the desired 3a-118 antibody.

Further, in embodiments, there is provided a cellular host that incorporates expressibly therein a polynucleotide that encodes the light chain, or the light chain variable region, of the misfolded human FasR antibody designated 3a-118, the polynucleotides having SEQ ID No. 101 (entire light chain) or SEQ ID No,102 In other embodiments, there is provided a cellular host that incorporates expressibly therein a polynucleotide that encodes the entire heavy chain SEQ ID No.99), or the heavy chain variable region (SEQ ID No.100), of the misfolded human FasR antibody designated 3a-118. In a further embodiment, there is provided a cellular host that incorporates expressibly therein both the heavy and light chain-encoding polynucleotides just recited, in either fully length form or in the form of variable

region-encoding polynucleotides. In a still further embodiment, there comprises the step of culturing the transfected cellular host, thereby to produce the desired 3a-118 antibody.

Also, in embodiments, there is provided a cellular host that incorporates expressibly therein a polynucleotide that encodes the light chain, or the light chain variable region, of the misfolded human FasR antibody designated 3d-19, the polynucleotides having SEQ ID No. 115 (entire light chain) or SEQ ID No,116, respectively. In other embodiments, there is provided a cellular host that incorporates expressibly therein a polynucleotide that encodes the heavy chain, or the heavy chain variable region, of the misfolded human FasR antibody designated 3d-19, having SEQ ID No. 113 (entire heavy chain) or SEQ ID No. 114 (heavy chain variable region). In a further embodiment, there is provided a cellular host that incorporates expressibly therein both the heavy and light chain-encoding polynucleotides just recited, in either fully length form or in the form of variable region-encoding polynucleotides. In a still further embodiment, there comprises the step of culturing the transfected cellular host, thereby to produce the desired 3d-19 antibody.

Further, in embodiments, there is provided a cellular host that incorporates expressibly therein a polynucleotide that encodes the light chain, or the light chain variable region, of the misfolded human FasR antibody designated 3d-19, the polynucleotides having SEQ ID No. 115 (entire light chain) or SEQ ID No,116 In other embodiments, there is provided a cellular host that incorporates expressibly therein a polynucleotide that encodes the entire heavy chain SEQ ID No.113), or the heavy chain variable region (SEQ ID No.114), of the misfolded human FasR antibody designated 3d-19. In a further embodiment, there is provided a cellular host that incorporates expressibly therein both the heavy and light chain-encoding polynucleotides just recited, in either fully length form or in the form of variable region-encoding polynucleotides. In a still further embodiment, there comprises the step of culturing the transfected cellular host, thereby to produce the desired 3d-19 antibody.

For use in the methods of the present invention, the antibodies and their binding fragments can be conjugated with other agents that are useful for the intended purpose, e.g., either diagnostic use or medical treatment. Agents appropriate for treating disease include cytotoxic agents or toxins that include chemotherapeutics and radiotherapeutics. For diagnostic purposes, appropriate agents are detectable labels that include radioisotopes or fluorescent markers for whole body imaging, and radioisotopes, enzymes, fluorescent labels and the like for sample testing.

For diagnostics, the detectable labels can be any of the various types used currently in the field of *in vitro* diagnostics, including particulate labels including biotin/streptavidin, metal sols such as colloidal gold, isotopes such as I125 or Tc99 presented for instance with a peptidic chelating agent of the N2S2, N3S or

N4 type, chromophores including fluorescent markers such as FITC and PE, luminescent markers, phosphorescent markers and the like, as well as enzyme labels that convert a given substrate to a detectable marker, and polynucleotide tags that are revealed following amplification such as by polymerase chain reaction. Suitable enzyme labels include horseradish peroxidase, alkaline phosphatase and the like. For instance, the label can be the enzyme alkaline phosphatase, detected by measuring the presence or formation of chemiluminescence following conversion of 1,2 dioxetane substrates such as adamantyl methoxy phosphoryloxy phenyl dioxetane (AMPPD), disodium 3-(4-(methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)tricyclo{3.3.1.1 3,7}decan}-4-yl) phenyl phosphate (CSPD), as well as CDP and CDP-star® or other luminescent substrates well-known to those in the art, for example the chelates of suitable lanthanides such as Terbium(III) and Europium(III). The detection means is determined by the chosen label. Appearance of the label or its reaction products can be achieved using the naked eye, in the case where the label is particulate and accumulates at appropriate levels, or using instruments such as a spectrophotometer, a luminometer, a fluorimeter, and the like, all in accordance with standard practice.

For therapy, the cytotoxin can be conjugated with the antibody or binding fragment through non-covalent interaction, but more desirably, by covalent linkage either directly or, more preferably, through a suitable linker. In a preferred embodiment, the conjugate comprises a cytotoxin and an antibody. Immunoconjugates of the antibody and cytotoxin are made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate, iminothiolane, bifunctional derivatives of imidoesters such as dimethyl adipimidate HCl, active esters such as disuccinimidyl suberate, aldehydes such as glutaraldehyde, bis-azido compounds such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates such as toluene 2,6-diisocyanate, and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). Carbon-14-labeled 1-isothiocyanobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is a chelating agent suitable for conjugation of radionuclide to the antibody.

The cytotoxin component of the immunoconjugate can be a chemotherapeutic agent, a toxin such as an enzymatically active toxin of bacterial, fungal, plant or animal origin such as urease, or fragments thereof, or a small molecule toxin, or a radioactive isotope such as 212Bi, 131I, 131In, 111In, 90Y, and 186Re, or any other agent that acts to inhibit the growth or proliferation of a senescent cell.

Chemotherapeutic agents useful in the generation of such immunoconjugates include maytansinoids including DM-1 and DM-4, adriamycin, doxorubicin, epirubicin, 5-fluoroouracil, cytosine arabinoside ("Ara-C"), cyclophosphamide, thiotepa, busulfan, cytoxin, taxoids, e.g. paclitaxel, and docetaxel, taxotere, methotrexate, cisplatin, melphalan, vinblastine, bleomycin, etoposide, ifosamide, mitomycin C,

mitoxantrone, vincristine, vinorelbine, carboplatin, teniposide, daunomycin, carminomycin, aminopterin, dactinomycin, mitomycins, esperamicins, 5-FU, 6-thioguanine, 6-mercaptopurine, actinomycin D, VP-16, chlorambucil, melphalan, and other related nitrogen mustards. Also included are hormonal agents that act to regulate or inhibit hormone action on tumors such as tamoxifen and onapristone. Toxins and fragments thereof which can be used include diphtheria A chain, nonbonding active fragments of diphtheria toxin, cholera toxin, botulinus toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, phytolaca Americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria, officinalis inhibitor, gelonin, saporin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothcenes. Small molecule toxins include, for example, calicheamicins, maytansinoids including DM-1 and DM-4, palytoxin and CC1065.

In an embodiment, the binding agent, optionally an antibody and/or binding fragment thereof, optionally conjugated to a toxin or a label or the nucleic acid is comprised in a composition. In an embodiment the composition comprises a diluent such as a saline solution for example phosphate buffered saline solution (0.05 – 1.0%).

The present invention also provides, for therapeutic use, a vaccine comprising any immunogenic form of an epitope that is unique to a misfolded form of a senescent cell surface protein, to treat subjects presenting with disease that is associated with senescent cells presenting that misfolded form of the protein. Such carriers will be nontoxic to recipients at the dosages and concentrations employed. Ordinarily, the preparation of such compositions entails combining the particular vaccine antigen with saline, buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose or dextrins, or chelating agents such as EDTA, glutathione and other stabilizers and excipients. Such compositions may be in suspension, emulsion or lyophilized form and will be pharmaceutically acceptable; i.e., suitably prepared and approved for use in the desired application. Preferred peptide vaccine compositions also comprise an adjuvant. DNA adjuvants are preferred for human use. The peptides may be formulated as fusions with other immunogenic peptides from the same or a different pathologic entity. Peptides may be synthesized as fusions of the epitopes identified herein with one or more T-helper epitope such as PADRE (SEQ I D NO: 118) or certain known tetanus peptides. Spacer peptides also may comprise part of these fusions. Materials having adjuvant activity are well known. Currently, however, Alum ($Al(OH)_3$), and similar aluminium gels are the only adjuvants licensed for human use. Other materials are also known to have adjuvant activity, and these include: Freund's complete adjuvant, a water-in-mineral-oil emulsion which contains killed, dried mycobacteria in the oil phase; Freund's incomplete adjuvant, a weaker formulation

without the mycobacteria; saponin, a membrane active glucoside extracted from the tree Quillia saponaria; nonionic block copolymer surfactants, non metabolised synthetic molecules which tend to bind proteins to cell surfaces; ISCOMS, lipid micelles incorporating Quil A (saponin) which mimic, in physical terms, infectious particles; and muramyl dipeptide, a leukocyte stimulatory molecule that is one of the active components of killed mycobacteria.

It will be appreciated that the vaccines noted above may comprise, instead of the epitope designated above, a variant thereof that incorporates 1, 2 or 3, amino acid additions, substitutions or deletions. Particularly the epitope may be a variant that has been truncated or extended to consist of 6, 7, or 8 amino acids, preferably 7 amino acids, and that incorporates up to 2, usually 1, amino acid substitution, for instance in which an amino acid is replaced by an oxidized form thereof, or an enantiomeric alternative thereof. It is apparent to those skilled in the art that substitution of certain amino acids in these epitopes will not affect immunoreactivity toward the epitopes. For example, substitution of leucine by isoleucine or valine and all combinations thereof is unlikely to alter the sensitivity of an antibody raised against this epitope. Thus all epitopes capable of generating antibodies reactive to the epitopes listed above for the purpose of selectively identifying misfolded specific protein are aspects of this invention.

It is occasionally desirable to derivatize amino acids present in the epitopes to obtain a more robust immune response or more selective reactivity toward the misfolded form. For example, a cysteine that on misfolding of its host protein may become oxidatively derivatized to cysteine sulfinic acid or cysteine sulfonic acid (cysteic acid). Thus antibodies against a free peptide containing, for example, a cysteic acid residue in place of cysteine are potentially more specific to the misfolded form of the protein. In general, candidate epitopes identified according to the methods described herein and containing derivatives of their constituent amino acids are an aspect of the present invention.

For epitopes containing proline, it may be desirable to prepare antigen peptides containing proline analogues that are fixed in the cis- or trans- configuration. Such analogues have been described previously (Scheraga et al, J Am Chem Soc 121 (49), 11558 (1999); Wang et al, J Org Chem 68 (6), 2343 (2002)). Unlike the other amino acids, for which there is a prohibitively large energy difference between the cis- and trans- amide bond stereoisomers, proline in unstructured peptides is able to interconvert between a cis- and trans- geometry on a relatively rapid time scale. When a proline is incorporated into the folded protein, steric interactions lock it into only one of the two possible conformers, but on unfolding it is free to racemerize. By raising antibodies against peptides incorporating a proline analogue with the opposite stereochemistry to that present in the native structure, the selectivity of the antibody for the unfolded state is much increased. Thus epitope peptides predicted by the method and incorporating

cis- or trans- analogues of proline are an aspect of this invention.

In embodiments of the present invention, vaccines useful in the treatment of disease include the following:

a) a vaccine that incorporates an immunogen comprising the epitope designated by SEQ ID No. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15, and particularly 1, 2, 5 and 9, for the depletion of senescent cells presenting misfolded PrP protein;

b) a vaccine that incorporates an immunogen comprising the epitope designated by SEQ ID No. 82, 83, 84, 85 and 86 for the depletion of senescent cells presenting misfolded CD38;

c) a vaccine that incorporates an immunogen comprising the epitope designated by SEQ ID No. 47, 48, 49, 50 or 51 for the depletion of senescent cells presenting misfolded CD44;

d) a vaccine that incorporates an immunogen comprising the epitope designated by SEQ ID No. 52, 53, 54, 55 or 56 for the depletion of senescent cells presenting misfolded Fas ligand;

e) a vaccine that incorporates an immunogen comprising the epitope designated by SEQ ID No. 57, 58, 59, 60, or 61 for the depletion of senescent cells presenting misfolded NOTCH1;

f) a vaccine that incorporates an immunogen comprising the epitope designated by SEQ ID No. 62, 63, 64,65, and 66, and preferably SEQ ID Nos. 62 and 65, for the depletion of senescent cells presenting misfolded Fas receptor; and

g) a vaccine that incorporates an immunogen comprising the epitope designated by SEQ ID No. 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80 and 81 for the depletion of senescent cells presenting misfolded EGFR.

In therapeutic use, the antibodies and corresponding fragments and conjugates that bind selectively to the senescent cell marker, and vaccines that elicit such antibodies, can be used to treat subjects presenting with or at risk for a disease associated with cell senescence including particularly the so-called aging diseases and conditions, including degenerative disease. The terms "treat", "treatment," "treating", "therapeutic use," or "treatment regimen" encompass prophylactic, palliative, and therapeutic modalities of administration of the compositions of the present invention, and include any and all uses of the present products that remedy a disease state, condition, symptom, sign, or disorder caused or associated with, either directly or indirectly, a senescent cell presenting a misfolded form of a protein, including an inflammation-based pathology, infectious disease, allergic response, hyperimmune response, or other

symptom to be treated, or which prevents, hinders, retards, or reverses the progression of symptoms, signs, conditions, or disorders associated therewith.

The term "subject" generally refers to mammals and other animals including humans and other primates, companion animals, zoo, and farm animals, including, but not limited to, cats, dogs, rodents, rats, mice, hamsters, rabbits, horses, cows, sheep, pigs, elk or other ungulates, goats, poultry, etc. A subject includes one who is to be tested, or has been tested for prediction, assessment or diagnosis of a disease or disorder associated with cell senescence. The subject may have been previously assessed or diagnosed using other methods, such as those in current clinical practice, or may be selected as part of a general population (a control subject). A subject may be a transgenic animal, e.g. a rodent, such as a mouse, that produces a target protein especially in misfolded form, or is lacking expression thereof (e.g. a 'knock-out' mouse). For example, the subject may a transgenic mouse overexpressing a normal form of the target protein or may be a wild-type mouse or hamster that has been infected with a misfolded form of the target protein.

For treatment, the binding agent, such as the immunogen used for active immunization and the antibody used for passive immunization are used in "effective amounts". These are amounts useful, in a treatment regimen, to reduce the effect of the senescent cells that present the misfolded protein target. It will be apparent that the present invention is applicable to a wide variety of diseases, and that the particular amount and treatment regimen effective to reduce the effect of the endogenous protein will vary with each disease or condition, in accordance with established clinical practice.

In addition to such vaccines, the present invention provides for the therapeutic use of binding agents such as antibodies in the treatment of subjects presenting with the conditions noted above, including conditions/diseases related by the presence of the given misfolded protein. For treatment, antibody that binds selectively to the target epitope is administered as a pharmaceutical composition, comprising the antibody and a pharmaceutically acceptable carrier, in dosage form.

Also provided in another aspect, is a pharmaceutical composition comprising a binding agent that is selectively toxic to senescent cells in an amount effective to reduce the number of such cells for use in depleting senescent cells endogenous to a subject, wherein the binding agent binds selectively to a senescent cell surface protein having a misfolded conformation.

Yet a further aspect includes a pharmaceutical composition comprising a binding agent that selectively binds selectively to a senescent cell surface protein having a misfolded conformation.

For antibodies, fragments and conjugates, the dosage form is optionally a liquid dosage form. Antibody solutions can be prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose or an

emulsifier such as polysorbate. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, DMSO and mixtures thereof with or without alcohol, and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. Conventional procedures and ingredients for the selection and preparation of suitable formulations are described, for example, in Remington's Pharmaceutical Sciences (2003 - 20th edition) and in The United States Pharmacopeia: The National Formulary (USP 24 NF19) published in 1999. Formulations optionally contain excipients including, but not limited to, a buffering agents, an anti-oxidant, a stabilizer, a carrier, a diluent, and an agent for pH adjustment. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersion and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl, or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins such as serum, albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN, PLURONICS or polyethylene glycol (PEG).

In treatment, the dose of antibody optionally ranges from about 0.0001 mg/kg to about 100 mg/kg, about 0.01 mg/kg to about 5 mg/kg, about 0.15 mg/kg to about 3 mg/kg, 0.5 mg/kg to about 2 mg/kg and about 1 mg/kg to about 2 mg/kg of the subject's body weight. In other embodiments the dose ranges from about 100 mg/kg to about 5 g/kg, about 500mg/kg to about 2mg/kg and about 750mg/kg to about 1.5 g/kg of the subject's body weight.

For example, depending on the type and severity of the disease, about 1 μg/kg to 15 mg/kg (e.g., 0.1-20 mg/kg) of antibody or conjugate is a candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage is in the range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. Unit doses can be in the range, for instance of about 5mg to 500mg, such as 50mg, 100mg,

150mg, 200mg, 250mg and 300mg. The progress of therapy is monitored by conventional techniques and assays.

Therapeutic use of an antibody according to the present invention entails antibody administration, by injection or infusion, to subjects presenting with a disease in which cells or fluids present the epitope targeted by the antibody, i.e., in which the misfolded target protein is present. Subjects that would benefit from treatment can be identified by their clinical features, together with examination of tissue samples or bodily fluids to identify cells that present the epitope targeted by the antibody, as discussed *infra*.

In embodiments of the present invention, antibody compositions useful in the treatment of disease include compositions that incorporate the following:

a) an antibody that binds selectively to the epitope designated by SEQ ID No. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15, and particularly 1, 2, 5 and 9, for the depletion of senescent cells presenting misfolded PrP protein;

b) an antibody that binds selectively to the epitope designated by SEQ ID No. 82, 83, 84, 85 and 86 for the depletion of senescent cells presenting misfolded CD38;

c) an antibody that binds selectively to the epitope designated by SEQ ID No. 47, 48, 49, 50 or 51 for the depletion of senescent cells presenting misfolded CD44;;

d) an antibody that binds selectively to the epitope designated by SEQ ID No. 52, 53, 54, 55 or 56 for the depletion of senescent cells presenting misfolded Fas ligand;

e) an antibody that binds selectively to the epitope designated by SEQ ID No. 57, 58, 59, 60, or 61 for the depletion of senescent cells presenting misfolded NOTCH1;

f) an antibody that binds selectively to the epitope designated by SEQ ID No. 62, 63, 64,65, and 66, and preferably SEQ ID Nos. 62 and 65, for the depletion of senescent cells presenting misfolded FasR; and

g) an antibody that binds selectively to the epitope designated by SEQ ID No. 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80 and 81 for the depletion of senescent cells presenting misfolded EGFR .

For treatment with a vaccine, subjects are immunized on a schedule that can vary from once a day, to once a week, to once a month, to once a year, to once a decade. A typical regimen includes an immunization followed by booster injections at 6 weekly intervals. Another regimen consists of immunization followed by booster injections 1, 2 and 12 months later. Alternatively, booster injections

will vary depending on the immune response and the physiological condition of the subject. For immunization, the epitope-containing immunogen can be administered in a dose that ranges from about 0.0001 microgram to 10 grams, about 0.01 microgram to about 1 gram, about 1 microgram to about 1 mg, and about 100 to 250 micrograms per treatment. In one embodiment the timing of administering treatment is at one or more of the following: 0 months, 2 months, 6 months, 9 months, and/or 12 months. In one regimen, the dosing is at 2, 6, 9, and 12 months following the first immunization. In another regimen, the dosing is at 2 and 4 weeks following the first immunization, and then monthly afterwards. In an alternative regimen, the dosing varies depending on the physiological condition of the subject and/or the response to the subject to prior immunizations. The route of administration optionally includes, but is not limited to, intramuscular and intraperitoneal injections. In one embodiment the composition is injected into the deltoid muscle.

The vaccine composition itself can further comprise adjuvants. Adjuvants for parenteral immunization include aluminum compounds (such as aluminum hydroxide, aluminum phosphate, and aluminum hydroxy phosphate). The antigen can be precipitated with, or adsorbed onto, the aluminum compound according to standard protocols. Other adjuvants such as RIBI (ImmunoChem, Hamilton, MT) can also be used in parenteral administration.

The active ingredients to be used for in vivo administration will be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations may be prepared. Suitable examples of sustained-release include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shapes articles, e.g., films or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly (2-hydroxyethyl-methacrylate), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate, and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37° C., resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions,

controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

Diagnostically useful compositions comprising the antibody will incorporate a carrier suitable for diagnostic purposes, such as a solution of saline or buffered saline including phosphate buffered saline, together with any desired stabilizers or preservatives. Of course, the composition can be provided in a lyophilized form to prolong storage stability.

In diagnostic use, the binding agent is exploited to detect the presence of senescent cells. The senescent cells to be detected may be in a sample obtained from any source, including a living subject. A subject includes one who is to be tested, or has been tested for prediction, assessment or diagnosis of a disease or disorder associated with a given misfolded protein target. The subject may have been previously assessed or diagnosed using other methods, such as those in current clinical practice, or may be selected as part of a general population (a control subject). A subject may be a transgenic animal, e.g. a rodent, such as a mouse, that produces a senescent cell presenting a target protein in misfolded form. For example, the subject may be a transgenic mouse overexpressing a normal form of the target protein or may be a wild-type mouse or other rodent that has been infected with a misfolded form of the target protein.

To assist with the identification of subjects who are candidates for treatment with the antibody or vaccine compositions of the invention, the present invention further provides for the detection of an epitope by *in vitro* or *in vivo* diagnostic methods.

To detect the presence of a misfolded protein in any given sample, the present invention provides a detection method in which a sample suspected to contain the misfolded protein is treated with an antibody or binding fragment that binds selectively to an epitope presented uniquely by the misfolded protein relative to the natively folded form of that protein; and determining whether an antigen:antibody complex has formed, the formation thereof being indicative of the presence in the sample of a misfolded form of said protein. In one embodiment, the epitope is one that has been identified by applying an epitope prediction method. In another embodiment, the epitope is one that is, or is comprised within, a peptide having any one of SEQ ID Nos.1-36 and 41-86.

In a related embodiment, the labeled antibodies of the invention, or labeled form of a binding fragment thereof, can be used *in vivo* to image the presence of the misfolded protein to which the antibody binds. To this end, the present invention provides an antibody or fragment in a form coupled to an agent useful for *in vivo* imaging, such as isotopes of technetium, gadolinium, and the like.

In situ detection of the binding to cancer cells bearing misfolded PrP can also be performed using the present antibody or fragment, by immunofluorescence or immunoelectron microscopy. For this purpose, a histological specimen is removed from the patient, and a labeled form of the present antibody is applied to it, preferably by overlaying the antibody on a biological sample, in keeping with standard immunohistochemistry techniques. This procedure also allows for distribution of the PrP antigen to be examined within biopsied tumour tissue, to reveal only those sites at which PrP is presented in misfolded form. It will be apparent for those skilled in the art that a wide variety of histological methods are readily available for in situ detection.

More particularly, antibodies or binding fragments of the present invention may be used to monitor the presence or absence of antibody reactivity in a biological sample, e.g., a tissue biopsy from brain, skin, liver, heart, kidney, pancreas, bowel, spleen, muscle, fat, skin, ovary and the like, from a cell, or from fluid such as cerebrospinal fluid, blood including plasma, urine, seminal fluid, and the like, using standard detection assays. Immunological assays may involve direct detection, and are particularly suited for screening large amounts of samples for the presence of senescent cells that present misfolded surface protein. For example, antibodies may be used in any standard immunoassay format (e.g., ELISA, Western blot, immunoprecipitation, flow cytometry or RIA assay) to measure complex formation. Any appropriate label which may be directly or indirectly visualized may be utilized in these detection assays including, without limitation, any radioactive, fluorescent, chromogenic (e.g., alkaline phosphatase or horseradish peroxidase), or chemiluminescent label, or hapten (for example, digoxigenin or biotin) which may be visualized using a labeled, hapten-specific antibody or other binding partner (e.g., avidin). Exemplary immunoassays are described, e.g., in Ausubel et al., supra, Harlow and Lane, Antibodies: A Laboratory Approach, Cold Spring Harbor Laboratory, New York (1988), and Moynagh and Schimmel, Nature 400:105, 1999. For example, using the antibodies described herein, misfolded PrP is readily detected at the cell surface using standard flow cytometry methods. Samples found to contain labeled complex compared to appropriate control samples are taken as indicating the presence of misfolded PrP, and are thus indicative of a disease state amenable to treatment with the present antibodies.

Senescent cell screening results that are obtained with misfolded protein binding agents can be confirmed using any other test appropriate for senescent cell detection. It is known, for instance that misfolded PrP is detectable not only on senescent cells, but also on certain cancer cells particularly including ovarian cancer cells. In certain instances, therefore, the present senescent cell detection method should be accompanied by a test that is confirmatory for senescent cells. In embodiments, the confirmatory test can be the established assay for B-galactosidase activity. It has been shown that senescent cells will use B-galactosidase as a substrate when cultured at mildly acidic pH, such as pH 6.0. As described by Dimri et

al in US 5795728, incorporated herein by reference, senescent cells can be identified by culturing cells in the presence of a B-galactosidase substrate, such as X-gal, and at pH 6.0. The cultured cells or tissue are then fixed in a solution such as 2% formaldehyde, 0.2% glutaraldehyde and PBS. The appearance of reaction products, revealed by staining, indicates that the cultured cell is senescent. Alternatively, any cell or tissue can be examined for senescence by assaying for the presence of INK4a/ARF expression, as described by Sharpless et al in US 8158347, also incorporated herein by reference. An elevation in this expression product indicates the tested cell is senescent. Of course, other methods are useful to identify senescent cells, including measurement of the incorporation of labeled DNA precursors such as 3H-thymidine and BrdU or measurement of cell markers that are expressed only in proliferating cells, such as PCNA or MTT. Senescent cells will test negative for these markers.

Such assays can also be performed prior to administering a binding agent or immunogen described herein and/or to identify subjects to be treated. In an embodiment, the method comprises detecting senescent cells in a sample containing cells to be screened according to a method described herein, for example using a binding agent, B-galactosidease assay etc or combinations thereof; and administering to the subject a binding agent that is selectively toxic to senescent cells in an amount effective to reduce the number of such cells, wherein the biding agent binds selectively to a senescent cell surface protein having a misfolded conformation, relative to said protein in a natively folded conformation. In an embodiment, the method comprises detecting senescent cells in a sample containing cells to be screened according to a method method described herein, for example using a binding agent, B-galactosidase assay etc or combinations thereof; administering to the subject a binding agent that binds selectively to a senescent cell surface protein having a misfolded conformation, for example to treat an age-related disease.

To detect the presence of a senescent cell in a given sample, the present invention provides a detection method in which a sample suspected to contain the senescent cell is treated with a binding agent, e.g., an antibody or binding fragment, that binds selectively to an epitope presented uniquely by the misfolded protein relative to the natively folded form of that protein; and determining whether an antigen:antibody complex has formed, the formation thereof being indicative of the presence in the sample of a senescent cell. In one embodiment, the epitope is one that is, or is comprised within, a peptide herein described.

When applied *in vitro*, the detection method entails analysis of a cell-containing sample of body fluid or tissue or organ from a subject, usually a subject suspected of having endogenous misfolded target protein. For example, the biological sample may a body fluid such as cerebrospinal fluid, blood, plasma, lymph fluid, serum, urine or saliva. A tissue or organ sample, such as that obtained from a solid or semi-solid tissue or organ, may be digested, extracted or otherwise rendered to a liquid form – examples of such

tissues or organs include cultured cells, blood cells, brain, neurological tissue, skin, liver, heart, kidney, pancreas, islets of Langerhans, bone marrow, blood, blood vessels, heart valve, lung, intestine, bowel, spleen, bladder, penis, face, hand, bone, muscle, fat, cornea or the like, including cancerous forms thereof. A biological sample or samples may be taken from a subject at any appropriate time, including before the subject is diagnosed with, or suspected of having a protein misfolding associated disease or disorder, during a therapeutic regimen for the treatment or amelioration of symptoms of that disease or disorder, after death of the subject (regardless of the cause, or suspected cause). Alternately, a biological sample may include donated body fluid or tissue, such as blood, plasma or platelets when in care of a centralized blood supply organization or institution.

The presence in the sample of a senescent cell presenting a misfolded target protein is confirmed if the antibody forms a detectable antigen:antibody complex. The formation of such complex can be determined using a wide variety of protocols that include ELISA, RIA, flow cytometry, Western blots, immunohistochemistry and the like. To reveal the complex and hence the presence of the epitope in the sample, the antibody desirably is provided as a labeled antibody by conjugation or coupling to an agent that is detectable either visually or with the aid of instrumentation. The agent, or label, is capable of producing, either directly or indirectly, a detectable signal. For example, the label may be radio-opaque or a radioisotope, such as 3H, 14C, 32P, 35S, 123I, 125I, 131I; a fluorescent (fluorophore) or chemiluminescent (chromophore) compound, such as fluorescein isothiocyanate, rhodamine or luciferin; an enzyme, such as alkaline phosphatase, beta-galactosidase or horseradish peroxidase; an imaging agent; or a metal ion. Alternatively, the epitope can be revealed using a labeled secondary reagent that binds to the epitope antibody, such as a labeled antibody that binds the epitope antibody, to reveal presence of the epitope indirectly. The presence of an antibody:antigen complex may be detected by indirect means that do not require the two agents to be in solution. For instance, the complex is detectable indirectly using flow cytometry, where the antibody binds to, and forms an antibody:antigen complex with, the epitope presented on the surface of an intact cell. The application of the antibodies for detection of cell-surface forms of the epitope is a very useful embodiment of the invention particularly for detection of senescent cells presenting such epitopes. Detection of such cells can be achieved using the well-established technique of flow cytometry. It will also be appreciated that the antigen:antibody complex can also be identified by non-antibody based methods, that include those which sort proteins based on size, charge and mobility, such as electrophoresis, chromatography, mass spectroscopy and the like.

In a related embodiment, the labeled antibodies of the invention, or labeled form of a binding fragment thereof, can be used in vivo to image the presence of senescent cells that present the misfolded protein to which the antibody binds. To this end, the present invention provides an antibody or fragment in a form

coupled to an agent useful for in vivo imaging, such as isotopes of technetium, gadolinium, and the like. In embodiments, the senescent cell detection method is conducted using antibodies that bind selectively to misfolded human PrP. In specific embodiments, the antibodies are selected from 1A1, c-120 and YYR MAbs, and misfolded PrP binding fragments and conjugates thereof. In other specific embodiments, the antibodies are selected from 3d-19 and 3a-118, and misfolded FasR-binding antibody fragments and conjugates thereof.

In therapeutic use, the binding agents that bind selectively to a misfolded protein marker on the surface of senescent cells can be used to treat patients or subjects presenting with or at risk for a disease associated with senescent cells. For therapeutic use, passive immunotherapy can be adopted by administering binding agents that are antibodies or binding fragments thereof. In the alternative, active immunotherapy can be adopted using vaccines that elicit the production of such antibodies.

For treatment, the active ingredient, such as the immunogen used for active immunization and the antibody used for passive immunization are used in "effective amounts". These are amounts useful, in a treatment regimen, to reduce the effect of the endogenous senescent cells by eliminating or reducing the number of senescent cells endogenous to the recipient. It will be apparent that the present invention is applicable to a wide variety of diseases, and that the particular amount and treatment regimen effective to reduce the effect of the endogenous protein will vary with each disease, in accordance with established clinical practice for each disease.

An anti-senescence therapeutic according to the invention may be administered with a pharmaceutically-acceptable diluent, carrier, or excipient, in unit dosage form.

Any appropriate route of administration can be employed, for example, parenteral, intravenous, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, or oral administration.

For the treatment of subjects presenting with senescent cells positive for misfolded target protein, the appropriate dosage of agent, e.g., an antibody, fragment or conjugate, will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the agent is administered for preventative or therapeutic purposes, previous therapy, the patients clinical history and response to the agent, and the discretion of the attending physician. The agent is suitably administered to the patient at one time or over a series of treatments, in accordance with dosing regimens discussed above.

Indications

It is anticipated that depletion of endogenous senescent cells will have a therapeutic effect and benefit on a variety of aging diseases and conditions, including particularly the degenerative disorders that accompany aging. More particularly, senescent cell depletion is expected to provide improvements in 1) reducing the rate at which adipose tissue is lost, 2) reducing the rate at which muscle fibre diameter is reduced, and 3) reducing the rate at which skin tone deteriorates over time. These effects are likely to be seen more dramatically in aged recipients, i.e. those at an age greater than 50 years, especially those aged greater than 60 years or more, such as 65 years, 70 years and 75 years and greater. Also, candidate recipients include those whose lifestyle imposes age-accelerating effects, including tobacco smokers and users, alcohol and narcotic drug abusers, skin tanning enthusiasts, and the like. These are therefore preferred recipients of the present treatment method.

Particular conditions and diseases that can be treated by the present method include sarcopenia. Sarcopenia is characterized first by a muscle atrophy (a decrease in the size of the muscle), along with a reduction in muscle tissue "quality," caused by such factors as replacement of muscle fibres with fat, an increase in fibrosis, changes in muscle metabolism, oxidative stress, and degeneration of the neuromuscular junction. Combined, these changes lead to progressive loss of muscle function and frailty.

Other conditions that can be treated by the present method include cataracts, and so-called "signs of aging" such as wrinkling and discolouration of the skin, and overall dermal tone. Treatment by the present method is expected to reduce the rate at which fat and muscle that support skin tone are reduced, so that skin wrinkling also is reduced, delayed or eliminated. As well treatment is expected to have a benefit on the rate at which cataracts form in the eye.

It has been demonstrated that senescent cells create a local environment that supports tumorigenesis in neighbouring cells. Treatment by the present method can accordingly be useful to treat cancer in various tissues, including cancers of the lung, prostate, skin, breast, and the like. Such treatment is expected to result in a decrease in the rate of tumour formation directly, and thus indirectly also on the number, size and distribution of responsive cancer cells and tumours.

In specific embodiments of the present invention, antibody compositions useful in the treatment of disease include compositions that incorporate the following:

a) an antibody that binds selectively to an epitope presented by peptides designated by SEQ ID No. 1-37 and 47-86 for the treatment of subjects afflicted with sarcopenia; and

b) an antibody that binds selectively to an epitope presented by peptides designated by SEQ ID No. 1-37 and 47-86 for the treatment of aged subjects presenting with signs of skin aging such as wrinkling, discolouration and the like; and

c) an antibody that binds selectively to an epitope presented by peptides designated by SEQ ID No. 1-37 and 47-86 for the treatment of aged subjects presenting with tumours.

The invention also includes articles of manufacture as well as kits that comprise components useful to perform the diagnostic and therapeutic methods of the present invention. The articles of manufacture comprise packaging material and a composition comprising an antibody or antisera that binds selectively to senescent cells that present an epitope unique to a misfolded form of protein, relative to the natively folded form of that protein. The composition includes a physiologically or pharmaceutically acceptable excipient, and the packaging material may include a label which indicates the active ingredients of the composition (e.g. the antisera or antibody). The label may further include an intended use of the composition, for example as a diagnostic reagent to be used with kits as set out herein.

Also provided is an article of manufacture, comprising packaging material and a composition comprising a peptide, or one or more peptides, as provided herein. The composition may include a physiologically or pharmaceutically acceptable excipient, and the packaging material may include a label which indicates the active ingredients of the composition (e.g. the peptide). The label may further include an intended use of the composition, for example as a therapeutic or prophylactic reagent, or as a composition to induce an immune response in a subject for the purpose of producing antisera or antibodies specific to senescent cell target protein, to be used with kits as set out herein.

In a further embodiment, there is provided a kit comprising a composition comprising one or more peptides as provided herein, along with instructions for use of the compound or composition for the production or screening of antibodies for identification of senescent cells. The kit may be useful for production and/or identification of senescent cell-specific antibodies or antisera, and the instructions may include, for example, dose concentrations, dose intervals, preferred administration methods, methods for immunological screening or testing, or the like.

In another embodiment, a kit for the preparation of a medicament, comprising a composition comprising one or more peptides as provided herein, along with instructions for its use is provided. The instructions may comprise a series of steps for the preparation of the medicament, the medicament being useful for inducing a therapeutic or prophylactic immune response in a subject to whom it is administered. The kit may further comprise instructions for use of the medicament in treatment for treatment, prevention or

amelioration of one or more symptoms of a disease or disorder associated with protein misfolding on senescent cells, or in which protein misfolding is implicated, and include, for example, dose concentrations, dose intervals, preferred administration methods or the like.

In another embodiment, a kit for diagnosing a disease or disorder associated with protein misfolding is provided. The kit comprises one or more misfolded protein-selective antibodies or antisera as described herein, along with instructions for its use. The antibody may further be coupled to a detection reagent. Examples of detection reagents include secondary antibodies, such as an anti-mouse antibody, an anti-rabbit antibody or the like. Such secondary antibodies may be coupled with an enzyme that, when provided with a suitable substrate, provides a detectable colorimetric or chemiluminescent reaction. The kit may further comprise reagents for performing the detection reaction, including enzymes such as proteinase K, blocking buffers, homogenization buffers, extraction buffers, dilution buffers or the like. The kit may further comprise reagents useful to perform the confirmatory B-galactosidase test for senescence, as discussed above.

In another embodiment, a kit for detecting the presence of senescent cells in a biological sample is provided. The kit comprises one or more antibodies or antisera that specifically bind the misfolded protein presented by the senescent cells, along with instructions for its use. The antibody may further be coupled to a detection reagent. Examples of detection reagents include secondary antibodies, such as an anti-mouse antibody, an anti-rabbit antibody or the like. Such secondary antibodies may be coupled with an enzyme that, when provided with a suitable substrate, provides a detectable colorimetric or chemiluminescent reaction. The kit may further comprise reagents for performing the detection reaction, including enzymes such as proteinase K, blocking buffers, homogenization buffers, extraction buffers, dilution buffers or the like. The kit may further comprise reagents useful to perform the confirmatory B-galactosidase test for senescence, as discussed above.

Example 1 – Antibodies to YYR epitope of misfolded human PrP (see US 7,041,807)

Epitopes uniquely exposed on misfolded, but not natively structured, PrPC reside in the peptides RYYRENMH and QVYYRPV (SEQ ID Nos 5 and 7 respectively). Resident within each of these peptides is the 3-amino acid epitope designated YYR noted above. Antibodies against the YYR epitope of PrP are described in the literature by Cashman (see US 7,041,807 incorporated herein by reference). Some of that work is reproduced below. As noted in the results, the antibodies to YYR bind selectively to the disease-misfolded form of PrPC, and do not bind to the natively folded form.

In order to develop an antibody to the YYR epitope presented by misfolded PrPC and but not by natively folded PrPC, a peptide with the amino acid sequence Acetyl-Cys-Tyr-Tyr-Arg-NH2 (YYR) was synthesized, conjugated to KLH, and injected intramuscularly into rabbits using well known techniques. At the amino-terminus of the peptide, a cysteine residue was added to allow conjugation of the peptide with the protein carrier. The amino group of the peptide was blocked by acetylation, and the carboxylic group of the peptide was blocked by amidation.

Peptides were synthesized using solid phase peptide synthesis methods either manually or automated (MPS396 peptides synthesizer, Advanced ChemTech). Coupling of amino acid residues was accomplished using Fmoc peptide synthesis chemistry (Fields et al., 1990, IJPPR 35, 161). Syntheses were performed on Wang or on amide Rink resins, with full side chain protection of amino acids. Since the alpha-NH2 groups of the amino acids were protected with the Fmoc group, the following protective groups were chosen for the side groups of the trifunctional amino acids:
Cysteine: 5-triphenylmethyl (Trt)
Arginine: 2,2,4,6,7-pentamethyldihydrobenzofuran-5 sulfonyl (Pbf)
Tyrosine: tert.-butyl ether (tBu)\
BOP, PyBOP, or TBTU were used as activation agents, depending on the chemistry and difficulty of the coupling reaction. All chemicals were purchased from Advanced Chem Tech, Bachem, and Calbiochem/NovaBiochem. Formation of each peptide bond between residues of the sequence was ensured by using a 3 to 6 fold excess of coupling reagents and by so-called double coupling; meaning that the coupling reaction was repeated for each amino acid added to the growing peptide chain.

After synthesis, the peptides were cleaved from the resin using the Reagent K as a cleavage mixture: water (2.5%), TIS (2.5%), EDT (2.5%), TFA (92.5%). The peptides were then precipitated with cold diethyl ether. The precipitates were centrifuged, washed three times with diethyl ether, dissolved in 20% - 50% AcCN/water mixture, and lyophilized. Analysis of crude products was performed using analytical RP-HPLC and electrospray MS.

The crude peptide was purified by Rp-HPLC (reverse phase high performance liquid chromatography) on a Vydac C18 column, 2.5x25 cm, using a linear gradient of 10-50% acetonitrile in water, with 0.06% TFA (1%/min gradient, 10 ml/min flow rate), with monitoring by UV at 215 nm and 254 nm. Analytical HPLC was used to estimate the purity of the fractions. The final product was obtained as a lyophilized peptide with at least 95% purity estimated by analytical HPLC (Vydac C18, 0.46x25 cm, linear gradient 10-60% acetonitrile in water, 0.1%TFA, 1%/min, 1ML/min flow rate, detection by UV absorption at 215

nm and 254 nm). The pure peptide was identified by molecular mass analysis using a SCIEX API III mass spectrometer according to standard procedures.

The retention time of the peptide on RP-HPLC was 21.215 minutes. The theoretical molecular weight of the peptide was calculated to be 644.74; the actual molecular weight, through molecular mass analysis, was found to be 646.5 (MW+H*).

Peptides were coupled to a carrier, in this case Keyhole limpet hemocyanin (KLH). Other carriers useful for such coupling include, without limitation, albumin, or ovalbumin, 8map, or lysozyme. Coupling was effected via a thioether linkage to the mercapto group of the cysteine. This type of linkage has the advantage that the peptide is coupled in a defined way to a carrier protein.

Coupling to KLH was performed as follows. 10mg of the peptide was dissolved in 2ml of phosphate buffered solution (PBS 1x). 1ml of KLH (pierce products #77100) was added to the peptide solution and stirred (1mole of peptide/50 amino acids). The KLH concentration was 10 mg/ml. 20 ul of glutaraldehyde (25% aqueous solution) was added to the peptide/carrier solution with constant stirring, incubated for 1 hour, after which a glycine stop solution was added. The peptide/carrier conjugate was separated from the peptide by dialysis against PBS

Polyclonal antibodies were prepared according to standard methods, and an immune response was enhanced with repeated booster injections, at intervals of 3 to 8 weeks. The success of the immunization was verified by determining the concentration of antibodies in a western blot or ELISA or both. More specifically, to generate polyclonal antibodies to misfolded PrPC (or PrPSc), the tripeptide YYR conjugated to KLH was injected into rabbits in accordance with a 164 day immunization regimen, after which the animals that had produced specific antibodies were bled.

In order to sample the serum prior to immunization, 10ml of blood per rabbit was taken as a preimmune control. Primary immunizations were carried out with Freund's complete adjuvant and subsequent boosts with incomplete Freund's adjuvant (IFA0 (1ml per rabbit, 0.5 ml per thigh muscle). Each injection consisted of approximately 200 ug of the purified peptide. At days 21,42 and 70, a booster injection was given with IFA. At days 31, 42 and 80, 10 ml of blood was collected from the central ear artery for titer determination (6 ml/kg/rabbit). At day 80, the titer of the sera was checked, and 3 more injections were given (IFA) at 4 week intervals, followed by blood sampling 10 days later. 10 days after the last boost, anesthetized rabbits were exsanguinated via cardiac puncture, and antisera were collected.

Goat polyclonal antibodies were generated according to standard methods. Three goats were immunized as follows. On day 1, all the goats received a primary immunization of 1 mg of YYR-KLH conjugates in complete Freund's adjuvant. Boosts were done by injection of 1 mg YYR-KLH in incomplete Freund's adjuvant for two of the three goats, whereas the third goat received 1mg YYR-8map conjugates in incomplete Freund's adjuvant. Serum samples from each of the three bleeds were tested for reactivity by ELISA against YYR-BSA conjugates. From the third set of bleeds, total lgG was purified by ammonium sulfate precipitation and YYR-reactive lgG was purified using a YYR affinity column. lgG fractions were tested for reactivity to PrPSc as described herein. The exact immunization schedule was as follows: Day 1, primary immunization; D 21, first boost immunization; Day 30, first bleed; Day 46, second boost immunization; Day 53, second boost immunization; Day 60, second bleed; Day 76, third boost immunization; Day 83, third boost immunization; and Day 90, third bleed.

Alternatively, monoclonal antibodies may be prepared using the synthetic peptides described herein and standard hybridoma technology(see, e.g., Kohler et al., Nature 256, 1975; Kohler et al., Eur. J. Immunol. 6:511, 1976; Kohler et al., Eur. J. Immunol. 6:292, 1976 Hammerling et al., In Monoclonal Antibodies and T Cell Hydridomas, Elsevier, NY, 1981; Ausubel et al., 1999, Current Protocols in Molecular Biology, Wiley Interscience, New York,) Once produced, monoclonal antibodies are also tested for specific PrP recognition by immunoprecipitation and western blot analysis.

The generation of monoclonal antibodies was carried out as follows. Mice were immunized with baculovirus supernatant containing mouse PrP-AP fusion protein in complete Freund's adjuvant, then boosted 2 weeks later with the same antigen in incomplete Freund's adjuvant. Two weeks after that immunization the mice were boosted with a mixture of PrP-AP supernatant plus 100 ug of KLH-CYYRRYYRYY (SEQ ID NO: 90 and 10 ug of KLH-CKYEDRYYRE (SEQ ID NO: 91) conjugates. Splenocytes from these mice were fused to the FO murine B cell line (ATCC CRL-1646) to generate specific hybridoma clones. Hybridoma supernatants were screened by ELISA. There were no reactive supernatants to PrP-AP or to the CKYEDRYYRE (SEQ ID NO: 91) sequence, although there were clones reactive to YYR-8map conjugates.

Total rabbit lgG was purified from serum using the Pharmacia protein A HiTrap column according to the manufacturer's recommendations. Briefly, a HiTrap column was equilibrated with 3 column volumes of start buffer (0.2M sodium phosphate buffer, pH7.0). Serum was applied, using a syringe through a luer adaptor, onto the column. The column was subsequently washed with 5 ml of start buffer. Bound protein

was eluted with 0.1M glycine, pH 3.0, and collected in eppendorf tubes containing 1M Tris pH 8.0 (50 ul/500 ul sample). Fractions were analyzed on SDS-PAGE.

Goat polyclonal antibodies were purified from serum samples as described above.

Mouse monoclonal antibodies were produced as ascites, and purified using a protein A column kit (Pierce) according to the manufacturer's instructions. Briefly, a sample of ascites was diluted with binding buffer at a 1:1 final ratio. The sample was then added to the top of the column, which had been previously equilibrated with binding buffer, and allowed to flow through the matrix. The pass-through material was collected and the column washed with 5 volumes of binding buffer. Mild elution buffer was added to the column to release the bound lgG antibody from the matrix. Other antibody isotypes were collected by switching to the lgG elution buffer. All the antibodies were collected in 1 ml fractions, which were analyzed by BCA to determine total protein content and SDS-PAGE electrophoresis to establish the degree of antibody purity. The fraction containing the most yield of lgG was desalted by passing it through a D-salt column (Pierce). The antibody fraction was allocated and stored at -80 C. in PBS

Antibodies produced using the aforementioned procedures were subsequently tested for high-affinity binding as follows.

Ten ul of brain extract was added to 950 ul of Immunoprecipitation buffer (PBS 3% NP-40, 3% Tween-20) and incubated at 37°C for 30 or 60 minutes. For experiments evaluating the reactivity of PrP 27-30 with the bead conjugates, the incubation was preceded by addition of 50 ul of 1 mg/ml proteinase K. Samples not treated with proteinase K were still incubated at 37 D. for the appropriate time period. After the incubation, 60 ul of a 100 mM PMSF solution were added to both sets of tubes. On hundred ul of resuspended bead conjugates were then added to the mixture, and incubated with rotation at room temperature for 2 hours. The beads were washed 3 times with washing buffer (PBS 2% NP-40 2% Tween-20) and resuspended by vortex after each wash. After the last wash, the beads were resuspended in 20 ul of 2x loading buffer (100mM Tris pH 6.8, 4% SDS, 0.015% bromphenol blue, 20% glycerol) and heated at 95°C for 3 minutes.

The PrPSc content of brain homogenates was determined by western blotting according to standard methods. Protein samples were mixed with 2x sample buffer at a ratio of 1:1 and boiled for 5 minutes at 100°C. SDS-PAGE analysis was performed according standard methods. Samples were applied to a pre-cast 15% acrylamide gels (Biorad) along with pre-stained molecular weight markers (Biorad). The gels were run at 100 V until the bromophenol blue dye front reached the bottom of the gel. The separated

protein was then transferred onto PVDF membranes at 100 V or 1 hr. The membranes were washed as described above before incubation with a goat anti-mouse lgG alkaline phosphatase conjugated secondary antibody (1:5000 in TBST) for 1 hour at room temperature. After washing, signals were developed with the chemiluminescent substrate CDP-star, and exposed to x-ray films.

Spleen cell suspensions were prepared from Balb/c mice by passing the tissues through a wire mesh. The cells were washed once with cold Dulbecco's PBS without Ca2+ or Mg2+ and viable cells were isolated by underlayering of the cell suspension with Lympholyte (Cedarlane) and centrifugation at 1300 g for 20 minutes. The cells were washed once with cold Dulbecco's PBS without CA2+ or Mg2+2.5% fetal bovine serum, and $0.5x10^6$ cells were aliquoted per well in a round bottom 96 well plate. The cells were centrifuged and resuspended in 50 ul of antibody-FITC conjugates at 1/10 final concentration in Dulbecco's PBS without CA2+ or Mg2+2.5% fetal bovine serum, for 15 minutes on ice. The cells were then washed twice with cold Dulbecco's PBS without Ca2+ or Mg2+2.5% fetal bovine serum and resuspended in the same medium containing 1 ug/ml of propidium iodide. The cells were analyzed on a Coulter Epics flow cytometer and were gated by size and granularity (forward and side scatter) and viability (exclusion of propidium iodide fluorescence).

Fluoresceinated mAbs were made by using the Fluorotag kit (Sigma) following the manufacturer's instructions. Briefly, 0.5 mg of each antibody was raised to pH 9 with concentrated bicarbonate buffer, and FITC stock solution was added to produce an FITC: antibody ratio of 20:1. The vials were then incubated for 2 hours at room temperature. Labeled antibody was separated from free FITC by passing the mixture over a Sephadex G-25M column. Conjugated antibodies were tested for successful fluoresceination by measuring their FITC emissions at 35 nm using an LJL Biosystems Analyst, and the antibodies were tested for retention of their binding activity with an ELISA against YYR-8map conjugates.

To determine whether antibody pAbC2 was useful in specifically recognizing PrPSc from bovine brain extracts, compared to PrPC using recombinant PrP (rbPrP), an ELISA approach was used. Either pools of PrPSc containing brain extracts or rbPrP was used to test the specificity of pAbC2 for PrPSc. The wells of an Immunolon ELISA plate (Dynex) were coated overnight at 4°C. with the PC2 containing culture supernatant in a TBS buffer containing 50mM Tris, pH 7.5, 150 mM NaC1, 1mM $CaC1_2$. For BSE-brain extract experiments, control wells were coated with a supernatant containing Mek-4; for rbPrP experiments, milk was used as a control to determine the non-specific binding of the antibody to the well. The coating of the ELISA plates with soluble PC2 was confirmed with an anti-PC2 monoclonal antibody. The wells were washed four times using a SLT 'Columbus' microplate washer (Tecan) with TBS

containing 0.05% Tween 20, and blocked by filling the wells with 0.2% I-Block (Tropix) in TBST and incubating the plate at 37°C for 1 hour. The plates were washed and the bovine brain homogenate (diluted to 1% w/v in TBS) or rbPrP was added to designated wells and incubated at RT for 1h. Wells were washed four times with TBST. pAbC2 was added to appropriate wells and incubated at RT or 1 hour, followed by a further 45 minute incubation with 100 ul of an anti-rabbit or mouse lgG/horseradish peroxidase conjugate (1:5000) in TBST containing 1% non-fat milk. Wells were washed four times with TBST. Signals were developed with TMB/H2O2 as a substrate for peroxidase. Reactions were stopped after 15 minutes by the addition of 100ul of 2M phosphoric acid. Signals were monitored at 450 nm with reference at 620 nm using a SLT microplate reader. Specific positive signals were determined by comparing PrP binding to PC2 with PrP binding to the negative control, Mek-4 or milk. Preimmune controls showed no binding.

It will thus be appreciated that various antibodies that recognize and bind selectively to the YYR epitope unique to misfolded PrPC can be obtained by raising antibodies against those epitopes in accordance with standard practices well established for these purposes.

Example 2 – Antibodies to YML epitope of misfolded human PrP (see WO 2010/099612)

As shown in Table 1, a region of PrPC beta strand 1 contains the epitope designated YML as shown by peptides LGGYML and GGYMLGS of SEQ ID Nos 1and 2, respectively. In the experiment that follows, the monoclonal IgM antibody 1A1 was raised specifically against the YML epitope using, as antigen/immunogen, a peptide having the sequence GGYMLGS (beta strand one with two flanking residues N and C terminal, SEQ ID No.2). This work is also described in WO 2010/099612, incorporated herein by reference.

General references: 263K hamster-adapted prions are described by Kimberlin et al., 1978. RML mouse-adapted prions are described by Chandler, R. L. (1961) (Lancet 1, 1378–1379). These may be used to infect mice or hamsters, using methods known in the art, for example those of Bueler H et al., 1993 (Cell 73:1339-1347), Oldstone et al., 2002, or Meade-White et al., 2009. Bolton et al., 1987 describe methods that may be used for isolation and purification of scrapie agent. Carlson et al., 1986 (Cell, 46:503-511) describes methods that may be used for clinical diagnosis of scrapie in mice and hamsters. Various transgenic mice overexpressing, partially expressing or lacking expression of PrP are described by Fischer et al., 1996 (EMBO J 15:1255-1264) and Weissmann et al., 2003 (British Medical Bulletin 66:43-60).

Brain tissues (normal and scrapie-infected mouse or hamster) were processed and analyzed as modified from Fischer et al 2000 (Nature 408:479-483). Briefly, 10% homogenates were made in PBS, 0.5%

deoxycholate (Sigma), 0.5% NP-40. The total protein concentration in the homogenate was determined by BCA assay (Pierce) and adjusted to 5mg/ml with homogenization buffer. For detection of PK-resistant material, 1.5 µl of homogenate was incubated with, or without, 0.15 µg PK (Sigma) at 37°C for 60 minutes. Digestion was halted by addition of 20 mM PMSF (Sigma).

Antibodies were conjugated to magnetic beads and used for immunoprecipitation experiments as modified from Paramithiotis, 2003. Briefly, $7x10^8$ magnetic beads (in 1ml PBS) (Dynal; Lake Success, New York) were coupled according to the manufacturer's instructions to: 1A1, 8B4, 4E4 or IgM isotype control. Conjugated beads were washed and blocked according to the manufacturer's recommendations, then resuspended in 1ml of PBS.

10 µL of antibody-coupled beads were incubated with 1 µL of 10% brain homogenate in 6% detergent (3% Tween20 and 3% NP40 in PBS) for 3hr at room temperature. Magnet-captured immune complexes were washed 3 times with 4% detergent (2% Tween20 and 2% NP40 in PBS), boiled in 4% SDS without reducing agents, and resolved on 15% Trisglycine or 4-12% bis-tris acrylamide gels (Invitrogen).

Immunoblotting was performed as described (Paramithiotis et al., 2003). Proteins were transferred onto PVDF membranes (Invitrogen). Membranes were blocked with 5% (w/v) dried non-fat milk. All incubations were done in TBST (25 mM Tris-HCl, 0.2 M NaCl, 0.5 % Tween-20). Peroxidase activity was detected by chemiluminescence; enhanced ECL (Amersham) or superWest Dura (Pierce; Rockford, Illinois).

Immunoprecipitated samples were analyzed by Western blotting with 6D11-biotin as the primary antibody (1:5000) and Strep-HRP as the secondary antibody (1:5000). 8B4-bead acted as a positive control and was able to immunoprecipitate PrP from all the brain homogenate samples except the PrP Knocked Out mouse (K/O). Beads only, IgM-isotype-beads and 4E4-beads acted as negative controls and as expected, no PrP was immunoprecipitated, except two very faint bands in the RML and 263K lanes as immunoprecipitated by the 4E4. 1A1, an IgM antibody that was raised against the beta-1 strand of PrP, was able to immunoprecipitate scrapie prion proteins from both RML (mouse scrapie strain) and 263K (hamster scrapie strain). There is a faint band in the Tg20 lane possibly due to a small content of misfolded PrP in this Prnp overexpressing transgenic mouse brain. Data indicated that 1A1 was able to recognize only the scrapie PrP, but not the wild type PrP in both mouse and hamster brain.

Example 3 - YML on the surface of tumors

The 1A1 antibody was tested for its ability to bind to both normal and tumor cells. As a control, the anti-PrPC antibody 6D11 was used to identify the level of expression of PrP on each cell type. The ability of

antibodies to bind to cells was observed by flow cytometry using HUVEC (human umbilical vein endothelial cells) as the normal cell type. Eight types of tumor cells were tested. Five of the cancer cells tested are immortalized cell lines from mice (B16 – melanoma, NSC34 – motor neuron/neuroblastoma hybrid) and humans (HL60 – promylocytic leukemia, MO3.13 – oligodendrocyte/muscle hybrid, SiHa – cervical carcinoma). The remaining cancer cells tested are primary tumor cells that have been propagated by the Living Tumor Laboratory (LTL) at the British Columbia Cancer Agency. Using proprietary technology, primary human tumors are propagated under the kidney capsules of immunodeficient mice. This allows the original tumor architecture and phenotype to remain consistent with the originally harvested tumor. The three tumors that have been tested for binding to the 1A1 antibody are LTL-013 (large diffuse B-Cell lymphoma), LTL-257 (colorectal sarcoma) and LTL-323 (melanoma).

By flow cytometry, the 1A1 YML monoclonal antibody shows minimal binding to normal HUVEC cells compared to an isotype IgM control. 1A1 also shows no detectable binding to HL60 myeloid leukemia compared to IgM isotype control. HUVECs and HL60 cells have moderate and high levels, respectively, of 6D11 immunoreactivity, indicating that YML exposure is not a simple function of prion protein expression. The 1A1 antibody also shows detectable binding to seven other human and mouse tumor cell lines and LTL human tumors, all of which also display detectable cell surface 6D11 prion protein immunoreactivity.

To determine if an anti-DSE antibody can modify tumor progression in vivo, the 1A1 antibody was tested for its ability to modify growth of a murine melanoma tumor (B16) in female C57Bl/6 mice. On day 0 of the study, 3 x 105 tumour cells were implanted subcutaneously into the flank of 12 mice. The mice were randomly assigned to two treatment groups. Group 1 was treated with PBS. Group 2 was treated with 1A1 antibody at 10 mg/kg. Mice were treated on days -1, 2 and 5.

Tumour growth was monitored by measuring tumour dimensions with calipers beginning on day 2. Tumour length and width measurements were obtained and tumour volumes were calculated according to the equation L x W2 /2 with the length (mm) being the longer axis of the tumour. Mice were sacrificed once tumour burden was high, according to standard animal care procedures. There is a significant difference in tumour growth between the two groups (paired t-test = 0.012; Wilcoxin = 0.007), indicating that a therapeutic effect of the 1A1 antibody has occurred.

Example 4 – Antibodies to the Rigid Loop epitope of misfolded human PrP

Peptides comprising the sequence MDEYSNQNN (SEQ ID No. 9) were synthesized using standard methods and then coupled to carrier proteins. Prepared immunogens included both KLH-Cys-

MEDYSNQNN and OVA-Cys-MDEYSNQNN. This work is described in the applicants co-pending USSN 61/658569 filed June 12, 2012 and incorporated herein by reference.

New Zealand white rabbits were immunized subcutaneously with 0.4 mg peptide-KLH conjugates in complete Freund's adjuvant. After the initial immunization, animals were boosted several times every 2-3 weeks. The rabbit with the best titer in immunoassay was intravenously boosted with peptide antigen again, four days before the removal of the spleen. The hybridoma fusion was performed using conventional PEG cell fusion methodology. Splenocytes were harvested from the immunized rabbit and fused with rabbit plasmacytoma cells 240E-W2 (US 5675063) using PEG4000 (Sigma Chemical, St. Louis, MO) and selected by HAT (hypoxanthine, aminopterin, and thymidine). At the end of selection hybridoma supernatants were collected and evaluated in various assays. Selected hybridomas were subsequently subcloned by limited dilution to obtain monoclonal hybridomas.

The antibody heavy and light chain genes for monoclonal ab120 were cloned from the hybridoma cells. Total RNA was extracted and reverse-transcribed to cDNA using the Qiagen TurboCapture mRNA kits. DNA fragments for L chain and the variable region (VH) of H chain of rabbit IgG were amplified by PCR with rabbit H and L chain primers. The L chain fragment was cloned into pTT5 mammalian expression vector and the VH fragment fused in-frame to the constant region of H chain pTT5 Heavy chain vector For each hybridoma clone, three plasmid DNA clones for H and L chains were sequenced and expressed as recombinant RabMAb for characterization.

Plasmids encoding the IgG heavy and light chains of ab120 were isolated from transformed E. coli using EndoFree® plasmid purification kit (Qiagen). Human HEK-293-6E cells were used for transient expression of ab120 antibody. The antibody plasmids were transfected into cells at logarithmic growth phase using FreeStyle™ MAX Reagent 293 fectin (Invitrogen, Cat: 51-0031) and cultured in FreeStyle™ 293 Expression Medium (Invitrogen, Cat: 12338-18) according to manufacturer's instructions. The transfected cells were grown at 37°C with 5% CO2 in an orbital shaker for 7 days. The antibody secreted into the culture medium was collected by spinning at 7000 rpm for 15 minutes to remove cell debris. The cleared culture supernatant was purified by protein A chromatography (HiTrapTM rProtein A FF, GE healthcare, CAT: 17-5080-01) under endotoxin free condition. Antibodies were eluted from the column in citrate elution buffer (SIGMA, CAT: C2404-100G) and adjusted to neutral pH with sodium bicarbonate buffer. The antibody preparation was concentrated and exchanged into PBS buffer. The concentration of IgG and endotoxin level in the final antibody preparation were determined by OD 280nm quantitation and Tachypleus Amebocyte Lysate gel clot assay (Zhanjiang A&C Biological Ltd), respectively.

Monoclonal antibody ab120 was purified by protein A. Purified antibody was filter-sterilized and stored at 4C in PBS buffer (pH 7.4). The protein concentration was determined by UV absorption 280 nm) assay and PBS buffer was used a blank buffer. The final concentration is the means from triplicate readings, and was given a QC requirement of > 2 mg/ml.

To measure protein purity, SDS-PAGE was performed with Bio-Rad mini electrophoresis system according to the manufacturer's instructions. The gel was then stained with Coomassie brilliant blue. The resolving gel was 12% acrylamide and the stacking gel was 4% acrylamide, with sample loading at 4 ug/lane. The assayed sample showed 2 bands (Heavy chain and Light chain) in reduced SDS-PAGE, and one band (whole IgG molecule) in non-reduced SDS-PAGE.

Endotoxin level was also assessed by the Gel Clot Tachypleus Ameboycte Lysate (TAL) kit using endotoxin standards and endotoxin-free water. Results indicated an endotoxin level of < 1EU/ml protein.

Thus in a preferred embodiment, the antibody is provided as a preparation that exhibits (a) < about 1 EU/ml protein, (b) a concentration of greater than about 2 mg/ml, (c) and migration as a single protein band when measured by non-reducing SDS-PAGE at a loading dose of 4ug/lane and detected at 280nm.

Maxisorp 96-well plates were coated overnight at 2-8°C with 100 ng/well of BSA-peptide in PBS. After blocking with PBST/casein, primary antibodies were added and incubated for 1 hour at room temperature. Rabbit antibodies were detected using goat anti-rabbit IgG-HRP and TMB substrate. After stopping the reaction with 0.25M sulfuric acid, absorbance was measured at 450 nm.

Recombinant PrP (Alicon) was mixed with LDS sample buffer (Life Technologies) and sample reducing agent (Life Technologies) and heated at 80°C for 20 minutes. After cooling for 15 minutes, Maxisorp 96-well plates were coated with 100 ng/well of denatured PrP and incubated at 2-8°C overnight. After blocking with PBST/BSA, primary antibodies were added and incubated for 1 hour at room temperature. Remaining steps were as described for anti-peptide ELISAs.

Maxisorp 96-well plates were coated overnight at 2-8°C with 100 ng/well of goat anti-His-6 antibody (QED) in PBS. After blocking with PBST/BSA, His-PrP (Alicon) was added and incubated for 1 hour at room temperature. Addition of primary antibody and remaining steps were as described for anti-peptide ELISAs.

Adherent tumor cell lines and primary cells were detached from flasks using non-enzymatic cell-dissociation buffer (Invitrogen). Peripheral blood mononuclear cells were prepared from fresh citrated blood on the day of collection using standard Ficoll centrifugation methods. Other primary cells were

frozen in 10% DMSO and thawed on the day of testing. Implanted tumors were surgically removed from mice. Tumors were chopped with scissors and then treated with collagenase/hyaluronidase (Worthington Biochemical) while shaking at 37°C for 30 minutes. Individual tumor cells were collected by passing the mixture through a 40μm screen.

Cells with Fc receptors were treated with 10% normal human serum to block the receptors. Cells were incubated with primary antibodies for 30 minutes at 2-8°C. Following washing, cells were incubated with goat anti-rabbit AF488 for 30 minutes at 2-8°C. After the final wash, cells were incubated in 1μg/mL propidium iodide. Cells were analyzed using either a Becton Dickinson FACSCalibur or a Becton Dickinson FACS Canto II and FCS Express Software (DeNovo Systems).

The Octet QK system from ForteBio was used (by sub-contractor T-mab). Measurements were performed according to standard protocols. BSA-peptides were biotinylated and then coupled to a streptavidin-coated biosensor. The specific antibodies in the hybridoma supernatants were allowed to incubate with the treated biosensor.

Binding of antibodies to denatured PrP was performed by ELISA as described above. Binding of antibodies to tumor cells was performed by FACS as described above. Antibodies were titrated to provide binding curves. EC50 values were calculated using GraphPad software.

RESULTS

The ProMis™ algorithm (described in WO 2010/040209) was used to identify DSEs for human PrP. DSE3 is called the rigid loop epitope, and it is located between β-sheet 2 and α-helix 1.

Anti-DSE3 antibodies were developed using the specific sequence MDEYSNQNN, and two different immunogens, i.e., KLH-Cys-DSE3 and OVA-Cys-DSE3. Rabbits were immunized as described in the Materials and Methods and the antisera from the rabbits were evaluated. Rabbits made excellent responses to the immunogen peptides. In addition, antisera showed excellent binding to full length denatured PrP. After performing fusions, monoclonal antibodies were generated. Seven recombinant rabbit monoclonal antibodies raised against DSE3 were then fully evaluated.

Antibodies were tested for binding to the immunogen peptides, and all seven antibodies showed excellent titers. Kd values for peptide binding were determined using surface plasmon resonance. All antibodies showed very high affinity for peptides, with Kds in the 10^{-10}M range.

All antibodies were then tested by ELISA for binding to denatured full-length recombinant PrP. One antibody exhibited a titer for denatured PrP that was similar to the anti-peptide affinities, in the 10^{-10} M

range. Thus, the preferred antibody exhibits preferably an EC50 by this test that is at least better than 10^{-9}M. The remaining antibodies showed logs lower affinity to denatured protein (10^{-6} to 10^{-9} M range) than to peptide. All antibodies were also tested by ELISA for binding to captured His-tagged PrP. None of the antibodies showed binding to captured His-PrP.

All seven antibodies were tested for binding to a panel of eleven tumor cell lines, six implanted primary human tumors, and nine normal cells. Only two antibodies showed binding to tumor cell lines (DSE3 ab90 and DSE3 ab120). When tested for binding to normal cells, DSE3 ab90 showed more binding to normal cells than to tumor cells. Although DSE3 ab120 also showed a small amount of binding to normal cells, this was less than the amount of binding observed against both tumor cell lines and passaged primary tumors. The binding of DSE ab120 was particularly strong against ovarian tumor cells , as the antibody bound well to five of six ovarian tumors tested, but did not bind to normal ovarian epithelial cells. PrP is expressed on all ovarian cells tested, although to varying degrees. In order to account for the differences in overall PrP levels, the binding of DSE3 ab120 was normalized to the binding of the control PrP antibody, 6H4.

For the six tumor cells, normalized DSE3 ab120 binding ranged from a low of 3.1 to a high of 71.6 (average = 18.8). However, the normalized DSE3 ab120 binding was only 0.4 for the normal ovarian cells.

In order to determine the affinity of DSE3 ab120 to tumor cells, antibody titrations were performed on three ovarian tumor cell lines. Antibody titrations were also performed on two types of normal cells, and confirmed the earlier findings that DSE3 ab120 does not bind to these normal cells. Even though up to 40 ug/mL of antibody was tested, binding saturation was not reached on the tumor cells and affinities could not be determined. In the same experiments, the PrP control antibody 6H4 was also titrated and binding saturation was reached. For 6H4, the average calculated EC50 is $1.7x10^{-8}$ M and there was no significant difference in the EC50 on tumor and normal cells. Since the binding of DSE3 ab120 to tumor cells is of lower affinity than 6H4, the EC50 for DSE3 ab120 must be lower than $1.7x10^{-8}$ M, and thus at least one log lower than the binding of DSE3 ab120 to denatured PrP ($8.6x10^{-10}$ M).

The location of the CDRs within the antibody is determined by numbering amino acid residues with reference to the Kabat numbering system.

This antibody, herein designated ab120, thus displays an affinity for binding to misfolded PrP when present on ovarian cancer cells. Its ability to detect senescent cells is explored in the next Example.

Example 5 – Senescent Cell Detection, PrP-Based

When primary cells, HUVECs are induced to undergo senescence, there is a proportion of cell surface PrP protein that stains for a misfolding-specific epitope referenced herein as the rigid loop.

More particularly, HUVEC cells were seeded at 50% confluence in 6-well culture plates and cultured in standard growth medium for 24 hours. Media was then removed and the cells were treated with 10µg/ml Mitomycin C for 2hrs in fresh cell culture medium. The solution was then removed and the cells were washed twice in PBS for about 30 seconds per wash. Lysosomal alkalinization was induced using the SA-β-gal staining kit, according to the supplier's instructions (Cell Biolabs, CBA-232). The cells were ultimately washed three times in PBS. A cell scraper was used to harvest the cells, and blocking with PBS containing 10% NGS for 30 minutes on ice. Those cells were then stained by first incubating the cells on ice with primary antibodies, i.e., antibody c-120 at 40µg/ml and antibody 6D11 as control at 5µg/ml, washing in cold PBS/2%NGS, and then incubating for 30 minutes on ice with secondary Alexa-488 labeled antibodies. Cells were then subjected to flow cytometry and analyzed. Results are shown in Figures 1 and 2.

When treated and untreated cells were incubated with either (1) a standard murine antibody (mIgG), (2) a PrP antibody that binds PrP in both natively and misfolded conformations (6D11), or (3) the c-120 PrP antibody that binds the rigid loop of PrP and thus binds selectively to PrP in a misfolded state, it was revealed (Figure 2) that senescent HUVECs were bound selectively by the misfolded PrP antibody.

Example 6 - Senescent Cell Detection, FasR-Based

The principles established herein are further demonstrated by targeting another and different cell surface protein first to determine if it, too, adopts a misfolded conformation when present on senescent cells, and then to determine if antibodies to that target can inhibit the growth of those target-positive cells.

Chosen as a second target was the protein FasR. Fas receptor (FasR) is known also as human tumour necrosis factor superfamily member 6 receptor (hTNFRSM6), and as CD95, and is implicated in cancer. It is a death receptor on the surface of of cells that leads to caspase-mediated programmed cell death (apoptosis). Antibodies to a misfolded form of this protein were prepared based on the epitopes predicted by Cashman et al, WO 2010/040209, as shown in Table 3:

Table 3

Human FASR (hTNFR6)	52-60	LHHDGQFCH	62

P25445 residues 1-335	70-80	ARDCTVNGDEP	63
	105-111	RLCDEGH	64
	136-142	NSTVCEH	65
	167-189	EEPSRSNLGWLCL	66

To raise antibodies against misfolded FasR, peptides comprising the FasR epitope sequences LHHDGQFCH (SEQ ID No. 62) and NSTVCEH (SEQ ID No. 65) were synthesized using standard methods and then coupled to carrier proteins. Prepared immunogens included both KLH-Cys-X and OVA-Cys-X, where X is NSTVCEH or LHHDGQFCH.

New Zealand white rabbits were immunized subcutaneously with 0.4 mg peptide-KLH conjugates in complete Freund's adjuvant. After the initial immunization, animals were boosted several times every 2-3 weeks. The rabbit with the best titer in immunoassay was intravenously boosted with peptide antigen again, four days before the removal of the spleen. The hybridoma fusion was performed using conventional PEG cell fusion methodology. Splenocytes were harvested from the immunized rabbit and fused with rabbit plasmacytoma cells 240E-W2 (US 5675063) using PEG4000 (Sigma Chemical, St. Louis, MO) and selected by HAT (hypoxanthine, aminopterin, and thymidine). At the end of selection hybridoma supernatants were collected and evaluated in various assays. Selected hybridomas were subsequently subcloned by limited dilution to obtain monoclonal hybridomas.

The antibody heavy and light chain genes for monoclonal AMF-3a-118 and AMF-3d-19 were cloned from the hybridoma cells. Total RNA was extracted and reverse-transcribed to cDNA using the Qiagen TurboCapture mRNA kits. DNA fragments for L chain and the variable region (VH) of H chain of rabbit IgG were amplified by PCR with rabbit H and L chain primers. The L chain fragment was cloned into pTT5 mammalian expression vector and the VH fragment fused in-frame to the constant region of H chain pTT5 Heavy chain vector For each hybridoma clone, three plasmid DNA clones for H and L chains were sequenced and expressed as recombinant RabMAb for characterization.

Plamids encoding the IgG heavy and light chains of AMF-3a-118 and AMF-3d-19 were isolated from transformed E. coli using EndoFree® plasmid purification kit (Qiagen). Human HEK-293-6E cells were used for transient expression of AMF-3a-118 and AMF-3d-19 antibodies. The antibody plasmids were transfected into cells at logarithmic growth phase using FreeStyle™ MAX Reagent 293 fectin (Invitrogen, Cat: 51-0031) and cultured in FreeStyle™ 293 Expression Medium (Invitrogen, Cat: 12338-18) according to manufacturer's instructions. The antibody secreted into the culture medium was collected by spinning at 7000 rpm for 15 minutes to remove cell debris. The cleared culture supernatant

was purified by protein A chromatography (HiTrapTM rProtein A FF, GE healthcare, CAT: 17-5080-01) under endotoxin free condition. Antibodies were eluted from the column in citrate elution buffer (SIGMA, CAT: C2404-100G) and adjusted to neutral pH with sodium bicarbonate buffer. The antibody preparations were concentrated and exchanged into PBS buffer.

Purified antibody was filter-sterilized and stored at 4°C in PBS buffer (pH 7.4). The protein concentration was determined by UV absorption 280 nm) assay and PBS buffer was used a blank buffer. To measure protein purity, SDS-PAGE was performed with Bio-Rad mini electrophoresis system according to the manufacturer's instructions. The gel was then stained with Coomassie brilliant blue. The resolving gel was 12% acrylamide and the stacking gel was 4% acrylamide. The assayed samples showed 2 bands (Heavy chain and Light chain) in reduced SDS-PAGE, and one band (whole IgG molecule) in non-reduced SDS-PAGE.

Thus in a preferred embodiment, the antibody is provided as a preparation that exhibits (a) a concentration of greater than about 1 mg/ml, (b) and migration as a single protein band when measured by non-reducing SDS-PAGE.

Maxisorp 96-well plates were coated overnight at 2-8°C with 100 ng/well of BSA-peptide in PBS. After blocking with PBST/casein, primary antibodies were added and incubated for 1 hour at room temperature. Rabbit antibodies were detected using goat anti-rabbit IgG-HRP and TMB substrate. After stopping the reaction with 0.25M sulfuric acid, absorbance was measured at 450 nm.

Recombinant Fas extracellular domain-Fc fusion protein (Aragen) was mixed with LDS sample buffer (Life Technologies) and sample reducing agent (Life Technologies) and heated at 80°C for 20 minutes. After cooling for 15 minutes, Maxisorp 96-well plates were coated with 100 ng/well of denatured Fas and incubated at 2-8°C overnight. After blocking with PBST/BSA, primary antibodies were added and incubated for 1 hour at room temperature. Remaining steps were as described for anti-peptide ELISAs.

His-tagged Fas (Creative Biomart) was mixed with Talon Dynabeads (Invitrogen) in round-bottom polystyrene 96-well plates for 30 minutes at room temperature. After beads were collected on a magnet, beads were washed 3x with PBST, then mixed with primary antibodies at room temperature for 60 minutes. Bound primary antibodies were detected with goat anti-rabbit IgG-HRP and TMB substrate. Supernatant was transferred to a flat-bottom 96-well plate (Nunc), the reaction stopped with 0.25M sulfuric acid, and absorbance was measured at 450 nm.

Antibodies having particularly good binding profiles include antibody AMF-3a-118 for the peptide LHHDGQFCH, and antibody AMF-3d-19 for the peptide NSTVCEH. These antibodies have the full

length protein sequences set out in SEQ ID No. 91 (light chain) and SEQ ID No.89 (heavy chain) for 3a-118, and sequences set out in SEQ ID No. 105 (light chain) and SEQ ID No.103 (heavy chain) for 3d-19. Polynucleotides encoding them have the sequences set out in SEQ ID No. 99 (heavy chain) and SEQ ID No.101 (light chain) for 3a-118 and SEQ ID No. 113 (heavy chain) and SEQ ID No. 115 (light chain) for antibody 3d-19. The important complementarity determining regions (CDRs) of these antibodies are set out below:

AMF-3a-118

For the heavy chain:

CDR1 (SEQ ID No. 93)
CDR2 (SEQ ID No. 94)
CDR3 (SEQ ID No. 95)

For the light chain:

CDR1 (SEQ ID No. 96)
CDR2 (SEQ ID No. 97)
CDR3 (SEQ ID No. 98)

AMF-3d-19

For the heavy chain:

CDR1 (SEQ ID No. 107)
CDR2 (SEQ ID No. 108)
CDR3 (SEQ ID No. 109)

For the light chain:

CDR1 (SEQ ID No. 110)
CDR2 (SEQ ID No. 111)
CDR3 (SEQ ID No. 112)

These misfolded FasR antibodies were assessed for their ability to detect senescent cells, using the approach already described above. More particularly, HUVEC cells were seeded at 50% confluence in 6-well culture plates and cultured in standard growth medium for 24 hours. Media was then removed and the cells were treated with 10μg/ml Mitomycin C for 2hrs in fresh cell culture medium. The solution was

then removed and the cells were washed twice in PBS for about 30 seconds per wash. Lysosomal alkalinization was induced using the SA-β-gal staining kit, according to the supplier's instructions (Cell Biolabs, CBA-232). The cells were ultimately washed three times in PBS. A cell scraper was used to harvest the cells, and blocking with PBS containing 10% NGS for 30 minutes on ice. Those cells were then stained by first incubating the cells on ice with primary antibodies, i.e., antibodies 3a-118 and 3d-19 at 40μg/ml and antibody 6D11 as control at 5μg/ml, washing in cold PBS/2%NGS, and then incubating for 30 minutes on ice with secondary Alexa-488 labeled antibodies. Cells were then subjected to flow cytometry and analyzed. Results are shown in Figures 3 and 4.

When treated and untreated cells were incubated with either (1) a standard murine antibody (mIgG), (2) a PrP antibody that binds PrP in both natively and misfolded conformations (6D11), or (3) the FasR antibodies that binds the noted exposed epitopes and thus bind selectively to FasR in a misfolded state, it was revealed (Figures 3 and 4) that senescent HUVECs were bound selectively by the misfolded FasR antibodies.

All citations are herein incorporated by reference, as if each individual publication was specifically and individually indicated to be incorporated by reference herein and as though it were fully set forth herein.

Table of Sequences

SEQ ID	Subject	Sequence
1	Misfolded PrP epitope	LGGYML
2	Misfolded PrP epitope	GGYMLGS
3	Misfolded PrP epitope	HFGSDYED
4	Misfolded PrP epitope	SDYED
5	Misfolded PrP epitope	RYYRENMH
6	Misfolded PrP epitope	RENMH
7	Misfolded PrP epitope	QVYYRPM
8	Misfolded PrP epitope	PMDEYSNQNN
9	Misfolded PrP epitope	MDEYSNQNN
10	Misfolded PrP epitope	KQHTVTTTTKGEN
11	Misfolded PrP epitope	ARDCTVNGDEP
12	Misfolded PrP epitope	RLCDEGH
13	Misfolded PrP epitope	NSTVCEH
14	Misfolded PrP epitope	EEPSRSNLGWLCL
15	Misfolded PrP sequence	RYYRE
16	Misfolded PrP sequence	RYYREN
17	Misfolded PrP sequence	RYYRENM
18	Misfolded PrP sequence	DRYYRENMH
19	Misfolded PrP sequence	DRYYRENM
20	Misfolded PrP sequence	VYYRPM
21	Misfolded PrP sequence	QVYYRP
22	Misfolded PrP sequence	QVYYR
23	Misfolded PrP sequence	QVYYRPMD
24	Misfolded PrP sequence	QVYYRPM
25	Misfolded PrP sequence	GGYMLG
26	Misfolded PrP sequence	GYMLGS
27	Misfolded PrP sequence	GGYML
28	Misfolded PrP sequence	YMLGS
29	Misfolded PrP sequence	GYML
30	Misfolded PrP sequence	YMLG
31	Misfolded PrP sequence	LGGYML
32	Misfolded PrP sequence	LGGYMLG
33	Misfolded PrP sequence	YML
34	Misfolded PrP sequence	PMDEYSNQNN
35	Misfolded PrP sequence	DEYSNQNN

36 Misfolded PrP sequence MDEYSNQ

37 Ab c-120 light chain

MDTRAPTQLLGLLLLWLPGATFAQVLTQTPSPVSAAVGGTVTINCQSSQSLYNKNWLSWYQKKPGQPPKLL
IYKASTLESGVSSRFKGSGSGTQFTLTISGVQCDDAATYYCQGEFSCSSADCTAFGGGTEVVVKGDPVAPT
VLIFPPAADQVATGTVTIVCVANKYFPDVTVTWEVDGTTQTTGIENSKTPQNSADCTYNLSSTLTLTSTQY
NSHKEYTCKVTQGTTSVVQSFNRGDC

38 Ab c-120 heavy chain

METGLRWLLLVAVLKGVQCQSVEESGGHLVTPGTPLTLTCTVSGIDLSTYAMGWVRQAPGKGLEWIGVITK
SGNTYYASWAKGRFAISKTSTTVDLKITSPTTEDTATYFCGRYGIGVSYYDIWGPGTLVTVSSGQPKAPSV
FPLAPCCGDTPSSTVTLGCLVKGYLPEPVTVTWNSGTLTNGVRTFPSVRQSSGLYSLSSVVSVTSSSQPVT
CNVAHPATNTKVDKTVAPSTCSKPTCPPPELLGGPSVFIFPPKPKDTLMISRTPEVTCVVVDVSQDDPEVQ
FTWYINNEQVRTARPPLREQQFNSTIRVVSTLPIAHQDWLRGKEFKCKVHNKALPAPIEKTISKARGQPLE
PKVYTMGPPREELSSRSVSLTCMINGFYPSDISVEWEKNGKAEDNYKTTPAVLDSDGSYFLYSKLSVPTSE
WQRGDVFTCSVMHEALHNHYTQKSISRSPGK

39 Ab c-120 light chain variable region (VL)

MDTRAPTQLLGLLLLWLPGATFAQVLTQTPSPVSAAVGGTVTINCQSSQSLYNKNWLSWYQKKPGQPPKLL
IYKASTLESGVSSRFKGSGSGTQFTLTISGVQCDDAATYYCQGEFSCSSADCTAFGGGTEVVV

40 Ab c-120 heavy chain variable region (VH)

METGLRWLLLVAVLKGVQCQSVEESGGHLVTPGTPLTLTCTVSGIDLSTYAMGWVRQAPGKGLEWIGVITK
SGNTYYASWAKGRFAISKTSTTVDLKITSPTTEDTATYFCGRYGIGVSYYDIWGPGTLVTVSSGQ

41	Ab c-120 CDR1 heavy	TYAMG
42	Ab c-120 CDR2 heavy	VITKSGNTYYASWAKG
43	Ab c-120 CDR3 heavy	YGIGVSYYDI
44	Ab c-120 CDR1 light	QSSQSLYNKNWLS
45	Ab c-120 CDR2 light	KASTLES
46	Ab c-120 CDR3 light	QGEFSCSSADCTA

47	misfolded CD44 epitope	NGRYSIS
48	misfolded CD44 epitope	EGHVV
49	misfolded CD44 epitope	TSNTSNYDT
50	misfolded CD44 epitope	NRDGTRYVQKG
51	misfolded CD44 epitope	ANNTFVYILTSNTSNYDT

52	misfolded hTNFR epitope	IHPQNNSICCT
53	misfolded hTNFR epitope	NDCPGPGQDTD
54	misfolded hTNFR epitope	YWSENLF
55	misfolded hTNFR epitope	HLSCQEKQN
56	misfolded hTNFR epitope	CTCHAGFFLRENECV
57	misfolded hNOTCH1 epitope	CEHAGKC
58	misfolded hNOTCH1 epitope	MPGYEGVHC
59	misfolded hNOTCH1 epitope	CLHNGRC
60	misfolded hNOTCH1 epitope	KINEF
61	misfolded hNOTCH1 epitope	ECASSPCLHNGRCLDKINEFQCECP
62	misfolded hFas epitope	LHHDGQFCH
63	misfolded hFas epitope	ARDCTVNGDEP
64	misfolded hFas epitope	RLCDEGH
65	misfolded hFas epitope	NSTVCEH
66	misfolded hFas epitope	EEPSRSNLGWLCL
67	misfolded hEGFR epitope	SNKLTQLFTFEDHFL
68	misfolded hEGFR epitope	VQRNYDLSFLK
69	misfolded hEGFR epitope	IRGNMYYENSYAL
70	misfolded hEGFR epitope	VLSNYDANKTG
71	misfolded hEGFR epitope	IVSSDFLSNMSMD
72	misfolded hEGFR epitope	FLSNMSMDFQNHLGS
73	misfolded hEGFR epitope	WGAGEE
74	misfolded hEGFR epitope	PPLMLYNPTTYQMDVNPE
75	misfolded hEGFR epitope	PEGKYSFGAT
76	misfolded hEGFR epitope	RNYVVTDHGS
77	misfolded hEGFR epitope	GADSYEMEEDGVRK
78	misfolded hEGFR epitope	NGIGIGEFKDSLSIN
79	misfolded hEGFR epitope	ATNIKHFKN
80	misfolded hEGFR epitope	LPVAFRGDSFTHTPPL
81	misfolded hEGFR epitope	KIISNRGENS
82	misfolded CD38 epitope	YTEIHPEMRHVDCQS
83	misfolded CD38 epitope	GEFATSKIN
84	misfolded CD38 epitope	WRKDCSN
85	misfolded CD38 epitope	GSRSKIFDKDS

86 misfolded CD38 epitope IHGGREDSRDL

87 DNA for ab c-120 heavy chain

ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAGTCGGTGGAGGAGTCCGG
GGGTCACCTGGTCACGCCTGGGACACCCCTGACACTCACCTGCACAGTCTCTGGAATCGACCTCAGTACCTATGCAA
TGGGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGGAGTCATTACTAAAAGTGGTAACACATACTAC
GCGAGCTGGGCGAAAGGCCGATTCGCCATCTCCAAAACCTCGACCACGGTGGATCTAAAGATCACCAGTCCGACAAC
CGAGGACACGGCCACCTATTTCTGTGGCAGATATGGTATTGGTGTTTCTTACTATGACATCTGGGGCCCAGGCACTC
TGGTCACCGTCTCCTCAGGGCAACCTAAGGCTCCATCAGTCTTCCCACTGGCCCCCTGCTGCGGGGACACACCCAGC
TCCACGGTGACCCTGGGCTGCCTGGTCAAAGGGTACCTCCCGGAGCCAGTGACCGTGACCTGGAACTCGGGCACCCT
CACCAATGGGGTACGCACCTTCCCGTCCGTCCGGCAGTCCTCAGGCCTCTACTCGCTGAGCAGCGTGGTGAGCGTGA
CCTCAAGCAGCCAGCCCGTCACCTGCAACGTGGCCCACCCAGCCACCAACACCAAAGTGGACAAGACCGTTGCGCCC
TCGACATGCAGCAAGCCCACGTGCCCACCCCCTGAACTCCTGGGGGGACCGTCTGTCTTCATCTTCCCCCCAAAACC
CAAGGACACCCTCATGATCTCACGCACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCAGGATGACCCCGAGG
TGCAGTTCACATGGTACATAAACAACGAGCAGGTGCGCACCGCCCGGCCGCCGCTACGGGAGCAGCAGTTCAACAGC
ACGATCCGCGTGGTCAGCACCCTCCCCATCGCGCACCAGGACTGGCTGAGGGGCAAGGAGTTCAAGTGCAAAGTCCA
CAACAAGGCACTCCCGGCCCCCATCGAGAAAACCATCTCCAAAGCCAGAGGGCAGCCCCTGGAGCCGAAGGTCTACA
CCATGGGCCCTCCCCGGGAGGAGCTGAGCAGCAGGTCGGTCAGCCTGACCTGCATGATCAACGGCTTCTACCCTTCC
GACATCTCGGTGGAGTGGGAGAAGAACGGGAAGGCAGAGGACAACTACAAGACCACGCCGGCCGTGCTGGACAGCGA
CGGCTCCTACTTCCTCTACAGCAAGCTCTCAGTGCCCACGAGTGAGTGGCAGCGGGGCGACGTCTTCACCTGCTCCG
TGATGCACGAGGCCTTGCACAACCACTACACGCAGAAGTCCATCTCCCGCTCTCCGGGTAAATGA

88 DNA coding for c-120 light chain

ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCCACATTTGCCCAAGTGCT
GACCCAGACTCCATCCCCTGTGTCTGCAGCTGTGGGAGGCACAGTCACCATCAATTGCCAGTCCAGTCAGAGTCTTT
ATAATAAGAACTGGTTATCCTGGTATCAGAAGAAACCAGGGCAGCCTCCTAAGCTCCTGATCTACAAGGCATCCACT
CTGGAATCTGGGGTCTCATCGCGGTTCAAGGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCGGCGTGCA
GTGTGACGATGCTGCCACTTACTACTGTCAAGGCGAATTTAGTTGTAGTAGTGCTGATTGTACGGCTTTCGGCGGAG
GGACCGAGGTGGTGGTCAAAGGTGATCCAGTTGCACCTACTGTCCTCATCTTCCCACCAGCTGCTGATCAGGTGGCA
ACTGGAACAGTCACCATCGTGTGTGTGGCGAATAAATACTTTCCCGATGTCACCGTCACCTGGGAGGTGGATGGCAC
CACCCAAACAACTGGCATCGAGAACAGTAAAACACCGCAGAATTCTGCAGATTGTACCTACAACCTCAGCAGCACTC
TGACACTGACCAGCACACAGTACAACAGCCACAAAGAGTACACCTGCAAGGTGACCCAGGGCACGACCTCAGTCGTC
CAGAGCTTCAATAGGGGTGACTGTTAG

89 AMF-3a-118 heavy chain

M E T G L R W L L L V A V L K G V Q C Q
S V E E S G G R L V T P G T P L T L T C
K A S G F S L S D S R V S W V R Q A P G
K G L E W I G I V G I G W N I Y H A N W
A K G R F T I S K T S S T T V D L K I T
S P T V E D T A T Y F C A R G L G G G T
V I W G P G T L V T V S L G Q P K A P S
V F P L A P C C G D T P S S T V T L G C
L V K G Y L P E P V T V T W N S G T L T
N G V R T F P S V R Q S S G L Y S L S S
V V S V T S S S Q P V T C N V A H P A T
N T K V D K T V A P S T C S K P T C P P
P E L L G G P S V F I F P P K P K D T L
M I S R T P E V T C V V V D V S Q D D P
E V Q F T W Y I N N E Q V R T A R P P L
R E Q Q F N S T I R V V S T L P I A H Q
D W L R G K E F K C K V H N K A L P A P
I E K T I S K A R G Q P L E P K V Y T M
G P P R E E L S S R S V S L T C M I N G
F Y P S D I S V E W E K N G K A E D N Y
K T T P A V L D S D G S Y F L Y S K L S
V P T S E W Q R G D V F T C S V M H E A
L H N H Y T Q K S I S R S P G K -

90 AMF-3a-118 heavy chain variable region

M E T G L R W L L L V A V L K G V Q C Q
S V E E S G G R L V T P G T P L T L T C
K A S G F S L S D S R V S W V R Q A P G
K G L E W I G I V G I G W N I Y H A N W
A K G R F T I S K T S S T T V D L K I T
S P T V E D T A T Y F C A R G L G G G T
V I W G P G T L V T V S L

91 AMF-3a-118 light chain

M D T R A P T Q L L G L L L L W L P G A
T F A Q V L T Q T P A S V S A A V G G T

V T I S C Q S S E S V Y K N N Y L S W F
Q Q K P G Q P P K L L I Y E A S K L A S
G V S T R F K G S G S G T Q F T L T I S
G V Q C D D A A T Y Y C L G E F S C Y S
G D C G T F G G G T A V V V K G D P V A
P T V L I F P P A A D Q V A T G T V T I
V C V A N K Y F P D V T V T W E V D G T
T Q T T G I E N S K T P Q N S A D C T Y
N L S S T L T L T S T Q Y N S H K E Y T
C K V T Q G T T S V V Q S F N R G D C -

92 AMF-3a-118 light chain variable region

M D T R A P T Q L L G L L L L W L P G A
T F A Q V L T Q T P A S V S A A V G G T
V T I S C Q S S E S V Y K N N Y L S W F
Q Q K P G Q P P K L L I Y E A S K L A S
G V S T R F K G S G S G T Q F T L T I S
G V Q C D D A A T Y Y C L G E F S C Y S
G D C G T F G G G T A V V V K

93 Ab 3a-118 CDR1 heavy DSRVS
94 Ab 3a-118 CDR2 heavy IVGIGWNIYHANWAKG
95 Ab 3a-118 CDR3 heavy GLGGGTVI
96 Ab 3a-118 CDR1 light QSSESVKNNYLS
97 Ab 3a-118 CDR2 light EASKLAS
98 Ab 3a-118 CDR3 light LGEFSCYSGDCGT

99 AMF-3a-118 heavy chain-encoding DNA

ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAGTCGGTGGAGGAGTCCGG
GGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGCAAAGCCTCTGGATTCTCCCTCAGTGACTCTAGAG
TGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAATCGTTGGCATTGGTTGGAATATATACCAC
GCGAACTGGGCGAAAGGCCGATTCACCATCTCCAAAACGTCGTCGACCACGGTGGATTTGAAAATCACCAGTCCGAC
AGTCGAGGACACGGCCACCTATTTCTGTGCCAGAGGTCTGGGTGGTGGTACTGTCATCTGGGGCCCAGGCACCCTGG
TCACCGTCTCCTTA
GGGCAACCTAAGGCTCCATCAGTCTTCCCACTGGCCCCCTGCTGCGGGGACACACCCAGCTCCACGGTGACCCTGGG
CTGCCTGGTCAAAGGGTACCTCCCGGAGCCAGTGACCGTGACCTGGAACTCGGGCACCCTCACCAATGGGGTACGCA

CCTTCCCGTCCGTCCGGCAGTCCTCAGGCCTCTACTCGCTGAGCAGCGTGGTGAGCGTGACCTCAAGCAGCCAGCCC
GTCACCTGCAACGTGGCCCACCCAGCCACCAACACCAAAGTGGACAAGACCGTTGCGCCCTCGACATGCAGCAAGCC
CACGTGCCCACCCCCTGAACTCCTGGGGGGGACCGTCTGTCTTCATCTTCCCCCCAAAACCCAAGGACACCCTCATGA
TCTCACGCACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCAGGATGACCCCGAGGTGCAGTTCACATGGTAC
ATAAACAACGAGCAGGTGCGCACCGCCCGGCCGCCGCTACGGGAGCAGCAGTTCAACAGCACGATCCGCGTGGTCAG
CACCCTCCCCATCGCGCACCAGGACTGGCTGAGGGGCAAGGAGTTCAAGTGCAAAGTCCACAACAAGGCACTCCCGG
CCCCCATCGAGAAAACCATCTCCAAAGCCAGAGGGCAGCCCCTGGAGCCGAAGGTCTACACCATGGGCCCTCCCCGG
GAGGAGCTGAGCAGCAGGTCGGTCAGCCTGACCTGCATGATCAACGGCTTCTACCCTTCCGACATCTCGGTGGAGTG
GGAGAAGAACGGGAAGGCAGAGGACAACTACAAGACCACGCCGGCCGTGCTGGACAGCGACGGCTCCTACTTCCTCT
ACAGCAAGCTCTCAGTGCCCACGAGTGAGTGGCAGCGGGGCGACGTCTTCACCTGCTCCGTGATGCACGAGGCCTTG
CACAACCACTACACGCAGAAGTCCATCTCCCGCTCTCCGGGTAAATGA

100 AMF-3a-118 heavy chain variable region-encoding DNA

ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAGTCGGTGGAGGAGTCCGG
GGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGCAAAGCCTCTGGATTCTCCCTCAGTGACTCTAGAG
TGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAATCGTTGGCATTGGTTGGAATATATACCAC
GCGAACTGGGCGAAAGGCCGATTCACCATCTCCAAAACGTCGTCGACCACGGTGGATTTGAAAATCACCAGTCCGAC
AGTCGAGGACACGGCCACCTATTTCTGTGCCAGAGGTCTGGGTGGTGGTACTGTCATCTGGGGCCCAGGCACCCTGG
TCACCGTCTCCTTA

101 AMF 3a-118 light chain-encoding DNA

ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCCACATTTGCCCAAGTGCT
GACCCAGACTCCAGCCTCCGTGTCTGCAGCTGTGGGAGGCACAGTCACCATCAGTTGCCAGTCCAGTGAGAGTGTTT
ATAAGAACAACTACTTATCCTGGTTTCAGCAGAAACCAGGACAGCCTCCCAAGCTCCTGATCTACGAAGCATCCAAA
CTGGCATCTGGGGTCTCAACGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCGGCGTGCA
GTGTGACGATGCTGCCACATACTACTGTCTAGGCGAATTTAGTTGTTATAGTGGTGATTGTGGTACTTTCGGCGGAG
GGACCGCGGTGGTGGTCAAA
GGTGATCCAGTTGCACCTACTGTCCTCATCTTCCCACCAGCTGCTGATCAGGTGGCAACTGGAACAGTCACCATCGT
GTGTGTGGCGAATAAATACTTTCCCGATGTCACCGTCACCTGGGAGGTGGATGGCACCACCCAAACAACTGGCATCG
AGAACAGTAAAACACCGCAGAATTCTGCAGATTGTACCTACAACCTCAGCAGCACTCTGACACTGACCAGCACACAG
TACAACAGCCACAAAGAGTACACCTGCAAGGTGACCCAGGGCACGACCTCAGTCGTCCAGAGCTTCAATAGGGGTGA
CTGTTAG

102 AMF 3a-118 light chain variable region-encoding DNA

ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCCACATTTGCCCAAGTGCT
GACCCAGACTCCAGCCTCCGTGTCTGCAGCTGTGGGAGGCACAGTCACCATCAGTTGCCAGTCCAGTGAGAGTGTTT
ATAAGAACAACTACTTATCCTGGTTTCAGCAGAAACCAGGACAGCCTCCCAAGCTCCTGATCTACGAAGCATCCAAA
CTGGCATCTGGGGTCTCAACGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCGGCGTGCA
GTGTGACGATGCTGCCACATACTACTGTCTAGGCGAATTTAGTTGTTATAGTGGTGATTGTGGTACTTTCGGCGGAG
GGACCGCGGTGGTGGTCAAA

103 AMF 3d-19 heavy chain

M E T G L R W L L L V A V L K G V Q C Q
S L E E S G G R L V T P G T P L T L T C
T V S G F S L S R N A I N W V R Q A P G
K G L E Y I G I I G S S G V T Y Y A S W
A K G R F T I S R T S T T V D L K I T S
P T T E D T A T Y F C A R N L Y T G G S
N D N L W G P G T L V T V S S G Q P K A
P S V F P L A P C C G D T P S S T V T L
G C L V K G Y L P E P V T V T W N S G T
L T N G V R T F P S V R Q S S G L Y S L
S S V V S V T S S S Q P V T C N V A H P
A T N T K V D K T V A P S T C S K P T C
P P P E L L G G P S V F I F P P K P K D
T L M I S R T P E V T C V V V D V S Q D
D P E V Q F T W Y I N N E Q V R T A R P
P L R E Q Q F N S T I R V V S T L P I A
H Q D W L R G K E F K C K V H N K A L P
A P I E K T I S K A R G Q P L E P K V Y
T M G P P R E E L S S R S V S L T C M I
N G F Y P S D I S V E W E K N G K A E D
N Y K T T P A V L D S D G S Y F L Y S K
L S V P T S E W Q R G D V F T C S V M H
E A L H N H Y T Q K S I S R S P G K -

104 AMF 3d-19 heavy chain variable region

M E T G L R W L L L V A V L K G V Q C Q
S L E E S G G R L V T P G T P L T L T C
T V S G F S L S R N A I N W V R Q A P G
K G L E Y I G I I G S S G V T Y Y A S W

A K G R F T I S R T S T T V D L K I T S
P T T E D T A T Y F C A R N L Y T G G S
N D N L W G P G T L V T V S S

105 AMF 3d-19 light chain

M D T R V P T Q L L G L L L L W L P G A
T F A Q V L T Q T P S P V S A A V G G T
V T I N C Q A S K S V Y N N V Q L S W F
Q Q K P G Q P P K R L I Y Y A S T L A S
G V P S R F K G S G S G T Q F T L T I S
D V Q C D D V A T Y Y C A G G Y S S S S
D N A F G G G T E V V V K G D P V A P T
V L I F P P A A D Q V A T G T V T I V C
V A N K Y F P D V T V T W E V D G T T Q
T T G I E N S K T P Q N S A D C T Y N L
S S T L T L T S T Q Y N S H K E Y T C K
V T Q G T T S V V Q S F N R G D C -

106 AMF 3d-19 light chain variable region

M D T R V P T Q L L G L L L L W L P G A
T F A Q V L T Q T P S P V S A A V G G T
V T I N C Q A S K S V Y N N V Q L S W F
Q Q K P G Q P P K R L I Y Y A S T L A S
G V P S R F K G S G S G T Q F T L T I S
D V Q C D D V A T Y Y C A G G Y S S S S
D N A F G G G T E V V V K

107 Ab 3d-19 CDR1 heavy RNAIN

108 Ab 3d-19 CDR2 heavy IIGSSGVTYYASWAKG

109 Ab 3d-19 CDR3 heavy NLYTGGSNDNL

110 Ab 3d-19 CDR1 light QASKSVYNNVQLS

111 Ab 3d-19 CDR2 light YASTLAS

112 Ab 3d-19 CDR3 light AGGYSSSSDNA

113 AMF 3d-19 heavy chain-encoding DNA

ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAGTCGTTGGAGGAGTCCGG
GGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGCACCGTCTCTGGATTCTCCCTCAGTCGCAATGCAA
TAAACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATACATCGGAATCATTGGTAGTAGTGGTGTCACATACTAC
GCGAGCTGGGCAAAAGGCCGATTCACCATCTCCAGAACCTCGACCACGGTGGATCTGAAAATCACCAGTCCGACAAC
CGAGGACACGGCCACCTATTTTTGTGCCAGAAATCTTTATACTGGTGGTAGTAATGATAACTTGTGGGGCCCAGGCA
CCCTGGTCACCGTCTCCTCA
GGGCAACCTAAGGCTCCATCAGTCTTCCCACTGGCCCCCTGCTGCGGGGACACACCCAGCTCCACGGTGACCCTGGG
CTGCCTGGTCAAAGGGTACCTCCCGGAGCCAGTGACCGTGACCTGGAACTCGGGCACCCTCACCAATGGGGTACGCA
CCTTCCCGTCCGTCCGGCAGTCCTCAGGCCTCTACTCGCTGAGCAGCGTGGTGAGCGTGACCTCAAGCAGCCAGCCC
GTCACCTGCAACGTGGCCCACCCAGCCACCAACACCAAAGTGGACAAGACCGTTGCGCCCTCGACATGCAGCAAGCC
CACGTGCCCACCCCCTGAACTCCTGGGGGGGACCGTCTGTCTTCATCTTCCCCCCAAAACCCAAGGACACCCTCATGA
TCTCACGCACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCAGGATGACCCCGAGGTGCAGTTCACATGGTAC
ATAAACAACGAGCAGGTGCGCACCGCCCGGCCGCCGCTACGGGAGCAGCAGTTCAACAGCACGATCCGCGTGGTCAG
CACCCTCCCCATCGCGCACCAGGACTGGCTGAGGGGCAAGGAGTTCAAGTGCAAAGTCCACAACAAGGCACTCCCGG
CCCCCATCGAGAAAACCATCTCCAAAGCCAGAGGGCAGCCCCTGGAGCCGAAGGTCTACACCATGGGCCCTCCCCGG
GAGGAGCTGAGCAGCAGGTCGGTCAGCCTGACCTGCATGATCAACGGCTTCTACCCTTCCGACATCTCGGTGGAGTG
GGAGAAGAACGGGAAGGCAGAGGACAACTACAAGACCACGCCGGCCGTGCTGGACAGCGACGGCTCCTACTTCCTCT
ACAGCAAGCTCTCAGTGCCCACGAGTGAGTGGCAGCGGGGCGACGTCTTCACCTGCTCCGTGATGCACGAGGCCTTG
CACAACCACTACACGCAGAAGTCCATCTCCCGCTCTCCGGGTAAATGA

114 AMF 3d-19 heavy chain variable region-encoding DNA

ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAGTCGTTGGAGGAGTCCGG
GGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGCACCGTCTCTGGATTCTCCCTCAGTCGCAATGCAA
TAAACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATACATCGGAATCATTGGTAGTAGTGGTGTCACATACTAC
GCGAGCTGGGCAAAAGGCCGATTCACCATCTCCAGAACCTCGACCACGGTGGATCTGAAAATCACCAGTCCGACAAC
CGAGGACACGGCCACCTATTTTTGTGCCAGAAATCTTTATACTGGTGGTAGTAATGATAACTTGTGGGGCCCAGGCA
CCCTGGTCACCGTCTCCTCA

115 AMF 3d-19 light chain-encoding DNA

ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAGTCGGTGGAGGAGTCCGG
GGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGCAAAGCCTCTGGATTCTCCCTCAGTGACTCTAGAG
TGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAATCGTTGGCATTGGTTGGAATATATACCAC
GCGAACTGGGCGAAAGGCCGATTCACCATCTCCAAAACGTCGTCGACCACGGTGGATTTGAAAATCACCAGTCCGAC

AGTCGAGGACACGGCCACCTATTTCTGTGCCAGAGGTCTGGGTGGTGGTACTGTCATCTGGGGCCCAGGCACCCTGG
TCACCGTCTCCTTA
GGGCAACCTAAGGCTCCATCAGTCTTCCCACTGGCCCCCTGCTGCGGGGACACACCCAGCTCCACGGTGACCCTGGG
CTGCCTGGTCAAAGGGTACCTCCCGGAGCCAGTGACCGTGACCTGGAACTCGGGCACCCTCACCAATGGGGTACGCA
CCTTCCCGTCCGTCCGGCAGTCCTCAGGCCTCTACTCGCTGAGCAGCGTGGTGAGCGTGACCTCAAGCAGCCAGCCC
GTCACCTGCAACGTGGCCCACCCAGCCACCAACACCAAAGTGGACAAGACCGTTGCGCCCTCGACATGCAGCAAGCC
CACGTGCCCACCCCCTGAACTCCTGGGGGGACCGTCTGTCTTCATCTTCCCCCCAAAACCCAAGGACACCCTCATGA
TCTCACGCACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCAGGATGACCCCGAGGTGCAGTTCACATGGTAC
ATAAACAACGAGCAGGTGCGCACCGCCCGGCCGCCGCTACGGGAGCAGCAGTTCAACAGCACGATCCGCGTGGTCAG
CACCCTCCCCATCGCGCACCAGGACTGGCTGAGGGGCAAGGAGTTCAAGTGCAAAGTCCACAACAAGGCACTCCCGG
CCCCCATCGAGAAAACCATCTCCAAAGCCAGAGGGCAGCCCCTGGAGCCGAAGGTCTACACCATGGGCCCTCCCCGG
GAGGAGCTGAGCAGCAGGTCGGTCAGCCTGACCTGCATGATCAACGGCTTCTACCCTTCCGACATCTCGGTGGAGTG
GGAGAAGAACGGGAAGGCAGAGGACAACTACAAGACCACGCCGGCCGTGCTGGACAGCGACGGCTCCTACTTCCTCT
ACAGCAAGCTCTCAGTGCCCACGAGTGAGTGGCAGCGGGGCGACGTCTTCACCTGCTCCGTGATGCACGAGGCCTTG
CACAACCACTACACGCAGAAGTCCATCTCCCGCTCTCCGGGTAAATGA

116 AMF 3d-19 light chain variable region-encoding DNA

ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAGTCGGTGGAGGAGTCCGG
GGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGCAAAGCCTCTGGATTCTCCCTCAGTGACTCTAGAG
TGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAATCGTTGGCATTGGTTGGAATATATACCAC
GCGAACTGGGCGAAAGGCCGATTCACCATCTCCAAAACGTCGTCGACCACGGTGGATTTGAAAATCACCAGTCCGAC
AGTCGAGGACACGGCCACCTATTTCTGTGCCAGAGGTCTGGGTGGTGGTACTGTCATCTGGGGCCCAGGCACCCTGG
TCACCGTCTCCTTA

119 DNA for ab c-120 heavy chain (lower case = non-coding restriction site)

aagcttgtacccttcacc
ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAGTCGGTGGAGGAGTCCGG
GGGTCACCTGGTCACGCCTGGGACACCCCTGACACTCACCTGCACAGTCTCTGGAATCGACCTCAGTACCTATGCAA
TGGGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGGAGTCATTACTAAAAGTGGTAACACATACTAC
GCGAGCTGGGCGAAAGGCCGATTCGCCATCTCCAAAACCTCGACCACGGTGGATCTAAAGATCACCAGTCCGACAAC
CGAGGACACGGCCACCTATTTCTGTGGCAGATATGGTATTGGTGTTTCTTACTATGACATCTGGGGCCCAGGCACTC
TGGTCACCGTCTCCTCAGGGCAACCTAAGGCTCCATCAGTCTTCCCACTGGCCCCCTGCTGCGGGGACACACCCAGC
TCCACGGTGACCCTGGGCTGCCTGGTCAAAGGGTACCTCCCGGAGCCAGTGACCGTGACCTGGAACTCGGGCACCCT
CACCAATGGGGTACGCACCTTCCCGTCCGTCCGGCAGTCCTCAGGCCTCTACTCGCTGAGCAGCGTGGTGAGCGTGA
CCTCAAGCAGCCAGCCCGTCACCTGCAACGTGGCCCACCCAGCCACCAACACCAAAGTGGACAAGACCGTTGCGCCC
TCGACATGCAGCAAGCCCACGTGCCCACCCCCTGAACTCCTGGGGGGACCGTCTGTCTTCATCTTCCCCCCAAAACC

CAAGGACACCCTCATGATCTCACGCACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCAGGATGACCCCGAGG
TGCAGTTCACATGGTACATAAACAACGAGCAGGTGCGCACCGCCCGGCCGCCGCTACGGGAGCAGCAGTTCAACAGC
ACGATCCGCGTGGTCAGCACCCTCCCCATCGCGCACCAGGACTGGCTGAGGGGCAAGGAGTTCAAGTGCAAAGTCCA
CAACAAGGCACTCCCGGCCCCCATCGAGAAAACCATCTCCAAAGCCAGAGGGCAGCCCCTGGAGCCGAAGGTCTACA
CCATGGGCCCTCCCCGGGAGGAGCTGAGCAGCAGGTCGGTCAGCCTGACCTGCATGATCAACGGCTTCTACCCTTCC
GACATCTCGGTGGAGTGGGAGAAGAACGGGAAGGCAGAGGACAACTACAAGACCACGCCGGCCGTGCTGGACAGCGA
CGGCTCCTACTTCCTCTACAGCAAGCTCTCAGTGCCCACGAGTGAGTGGCAGCGGGGCGACGTCTTCACCTGCTCCG
TGATGCACGAGGCCTTGCACAACCACTACACGCAGAAGTCCATCTCCCGCTCTCCGGGTAAATGA
gcgctgtgccggcgagctgcggccgc

120 DNA coding for c-120 light chain (lower case = non-coding restriction site)

aagcttgtacccttcacc
ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCCACATTTGCCCAAGTGCT
GACCCAGACTCCATCCCCTGTGTCTGCAGCTGTGGGAGGCACAGTCACCATCAATTGCCAGTCCAGTCAGAGTCTTT
ATAATAAGAACTGGTTATCCTGGTATCAGAAGAAACCAGGGCAGCCTCCTAAGCTCCTGATCTACAAGGCATCCACT
CTGGAATCTGGGGTCTCATCGCGGTTCAAGGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCGGCGTGCA
GTGTGACGATGCTGCCACTTACTACTGTCAAGGCGAATTTAGTTGTAGTAGTGCTGATTGTACGGCTTTCGGCGGAG
GGACCGAGGTGGTGGTCAAAGGTGATCCAGTTGCACCTACTGTCCTCATCTTCCCACCAGCTGCTGATCAGGTGGCA
ACTGGAACAGTCACCATCGTGTGTGTGGCGAATAAATACTTTCCCGATGTCACCGTCACCTGGGAGGTGGATGGCAC
CACCCAAACAACTGGCATCGAGAACAGTAAAACACCGCAGAATTCTGCAGATTGTACCTACAACCTCAGCAGCACTC
TGACACTGACCAGCACACAGTACAACAGCCACAAAGAGTACACCTGCAAGGTGACCCAGGGCACGACCTCAGTCGTC
CAGAGCTTCAATAGGGGTGACTGTTAG
agtgagagcggccgc

121 AMF-3a-118 heavy chain-encoding DNA (sequence before ATG and after TGA, vector sequence; underlined sequence restriction site)

*AAGCTT*GTACCCTTCACC
ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAGTCGGTGGAGGAGTCCGG
GGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGCAAAGCCTCTGGATTCTCCCTCAGTGACTCTAGAG
TGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAATCGTTGGCATTGGTTGGAATATATACCAC
GCGAACTGGGCGAAAGGCCGATTCACCATCTCCAAAACGTCGTCGACCACGGTGGATTTGAAAATCACCAGTCCGAC
AGTCGAGGACACGGCCACCTATTTCTGTGCCAGAGGTCTGGGTGGTGGTACTGTCATCTGGGGCCCAGGCACCCTGG
TCACCGTCTCCTTA
GGGCAACCTAAGGCTCCATCAGTCTTCCCACTGGCCCCCTGCTGCGGGGACACACCCAGCTCCACGGTGACCCTGGG
CTGCCTGGTCAAAGGGTACCTCCCGGAGCCAGTGACCGTGACCTGGAACTCGGGCACCCTCACCAATGGGGTACGCA

CCTTCCCGTCCGTCCGGCAGTCCTCAGGCCTCTACTCGCTGAGCAGCGTGGTGAGCGTGACCTCAAGCAGCCAGCCC
GTCACCTGCAACGTGGCCCACCCAGCCACCAACACCAAAGTGGACAAGACCGTTGCGCCCTCGACATGCAGCAAGCC
CACGTGCCCACCCCCTGAACTCCTGGGGGGACCGTCTGTCTTCATCTTCCCCCCAAAACCCAAGGACACCCTCATGA
TCTCACGCACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCAGGATGACCCCGAGGTGCAGTTCACATGGTAC
ATAAACAACGAGCAGGTGCGCACCGCCCGGCCGCCGCTACGGGAGCAGCAGTTCAACAGCACGATCCGCGTGGTCAG
CACCCTCCCCATCGCGCACCAGGACTGGCTGAGGGGCAAGGAGTTCAAGTGCAAAGTCCACAACAAGGCACTCCCGG
CCCCCATCGAGAAAACCATCTCCAAAGCCAGAGGGCAGCCCCTGGAGCCGAAGGTCTACACCATGGGCCCTCCCCGG
GAGGAGCTGAGCAGCAGGTCGGTCAGCCTGACCTGCATGATCAACGGCTTCTACCCTTCCGACATCTCGGTGGAGTG
GGAGAAGAACGGGAAGGCAGAGGACAACTACAAGACCACGCCGGCCGTGCTGGACAGCGACGGCTCCTACTTCCTCT
ACAGCAAGCTCTCAGTGCCCACGAGTGAGTGGCAGCGGGGCGACGTCTTCACCTGCTCCGTGATGCACGAGGCCTTG
CACAACCACTACACGCAGAAGTCCATCTCCCGCTCTCCGGGTAAATGA
GCGCTGTGCCGGCGAGCT*GCGGCCGC*

122 AMF 3a-118 light chain-encoding DNA (sequence before ATG and after TAG, vector sequence; underlined sequence restriction site)

aagcttgtacccttcacc
ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCCACATTTGCCCAAGTGCT
GACCCAGACTCCAGCCTCCGTGTCTGCAGCTGTGGGAGGCACAGTCACCATCAGTTGCCAGTCCAGTGAGAGTGTTT
ATAAGAACAACTACTTATCCTGGTTTCAGCAGAAACCAGGACAGCCTCCCAAGCTCCTGATCTACGAAGCATCCAAA
CTGGCATCTGGGGTCTCAACGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCGGCGTGCA
GTGTGACGATGCTGCCACATACTACTGTCTAGGCGAATTTAGTTGTTATAGTGGTGATTGTGGTACTTTCGGCGGAG
GGACCGCGGTGGTGGTCAAA
GGTGATCCAGTTGCACCTACTGTCCTCATCTTCCCACCAGCTGCTGATCAGGTGGCAACTGGAACAGTCACCATCGT
GTGTGTGGCGAATAAATACTTTCCCGATGTCACCGTCACCTGGGAGGTGGATGGCACCACCCAAACAACTGGCATCG
AGAACAGTAAAACACCGCAGAATTCTGCAGATTGTACCTACAACCTCAGCAGCACTCTGACACTGACCAGCACACAG
TACAACAGCCACAAAGAGTACACCTGCAAGGTGACCCAGGGCACGACCTCAGTCGTCCAGAGCTTCAATAGGGGTGA
CTGTTAG
Agtgaga*gcggccgc*

123 AMF 3d-19 heavy chain-encoding DNA (sequence before ATG and after TGA, vector sequence; underlined sequence restriction site)

*aagctt*gtacccttcacc
ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAGTCGTTGGAGGAGTCCGG
GGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGCACCGTCTCTGGATTCTCCCTCAGTCGCAATGCAA
TAAACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATACATCGGAATCATTGGTAGTAGTGGTGTCACATACTAC
GCGAGCTGGGCAAAAGGCCGATTCACCATCTCCAGAACCTCGACCACGGTGGATCTGAAAATCACCAGTCCGACAAC

CGAGGACACGGCCACCTATTTTTGTGCCAGAAATCTTTATACTGGTGGTAGTAATGATAACTTGTGGGGCCCAGGCA
CCCTGGTCACCGTCTCCTCA
GGGCAACCTAAGGCTCCATCAGTCTTCCCACTGGCCCCCTGCTGCGGGGACACACCCAGCTCCACGGTGACCCTGGG
CTGCCTGGTCAAAGGGTACCTCCCGGAGCCAGTGACCGTGACCTGGAACTCGGGCACCCTCACCAATGGGGTACGCA
CCTTCCCGTCCGTCCGGCAGTCCTCAGGCCTCTACTCGCTGAGCAGCGTGGTGAGCGTGACCTCAAGCAGCCAGCCC
GTCACCTGCAACGTGGCCCACCCAGCCACCAACACCAAAGTGGACAAGACCGTTGCGCCCTCGACATGCAGCAAGCC
CACGTGCCCACCCCCTGAACTCCTGGGGGGACCGTCTGTCTTCATCTTCCCCCCAAAACCCAAGGACACCCTCATGA
TCTCACGCACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCAGGATGACCCCGAGGTGCAGTTCACATGGTAC
ATAAACAACGAGCAGGTGCGCACCGCCCGGCCGCCGCTACGGGAGCAGCAGTTCAACAGCACGATCCGCGTGGTCAG
CACCCTCCCCATCGCGCACCAGGACTGGCTGAGGGGCAAGGAGTTCAAGTGCAAAGTCCACAACAAGGCACTCCCGG
CCCCCATCGAGAAAACCATCTCCAAAGCCAGAGGGCAGCCCCTGGAGCCGAAGGTCTACACCATGGGCCCTCCCCGG
GAGGAGCTGAGCAGCAGGTCGGTCAGCCTGACCTGCATGATCAACGGCTTCTACCCTTCCGACATCTCGGTGGAGTG
GGAGAAGAACGGGAAGGCAGAGGACAACTACAAGACCACGCCGGCCGTGCTGGACAGCGACGGCTCCTACTTCCTCT
ACAGCAAGCTCTCAGTGCCCACGAGTGAGTGGCAGCGGGGCGACGTCTTCACCTGCTCCGTGATGCACGAGGCCTTG
CACAACCACTACACGCAGAAGTCCATCTCCCGCTCTCCGGGTAAATGA
Gcgctgtgccggcgagct*gcggccgc*

124 AMF 3d-19 light chain-encoding DNA (sequence before ATG and after TGA, vector sequence; underlined sequence restriction site)

*aagctt*gtacccttcacc
ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAGTCGGTGGAGGAGTCCGG
GGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGCAAAGCCTCTGGATTCTCCCTCAGTGACTCTAGAG
TGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAATCGTTGGCATTGGTTGGAATATATACCAC
GCGAACTGGGCGAAAGGCCGATTCACCATCTCCAAAACGTCGTCGACCACGGTGGATTTGAAAATCACCAGTCCGAC
AGTCGAGGACACGGCCACCTATTTCTGTGCCAGAGGTCTGGGTGGTGGTACTGTCATCTGGGGCCCAGGCACCCTGG
TCACCGTCTCCTTA
GGGCAACCTAAGGCTCCATCAGTCTTCCCACTGGCCCCCTGCTGCGGGGACACACCCAGCTCCACGGTGACCCTGGG
CTGCCTGGTCAAAGGGTACCTCCCGGAGCCAGTGACCGTGACCTGGAACTCGGGCACCCTCACCAATGGGGTACGCA
CCTTCCCGTCCGTCCGGCAGTCCTCAGGCCTCTACTCGCTGAGCAGCGTGGTGAGCGTGACCTCAAGCAGCCAGCCC
GTCACCTGCAACGTGGCCCACCCAGCCACCAACACCAAAGTGGACAAGACCGTTGCGCCCTCGACATGCAGCAAGCC
CACGTGCCCACCCCCTGAACTCCTGGGGGGACCGTCTGTCTTCATCTTCCCCCCAAAACCCAAGGACACCCTCATGA
TCTCACGCACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCAGGATGACCCCGAGGTGCAGTTCACATGGTAC
ATAAACAACGAGCAGGTGCGCACCGCCCGGCCGCCGCTACGGGAGCAGCAGTTCAACAGCACGATCCGCGTGGTCAG
CACCCTCCCCATCGCGCACCAGGACTGGCTGAGGGGCAAGGAGTTCAAGTGCAAAGTCCACAACAAGGCACTCCCGG
CCCCCATCGAGAAAACCATCTCCAAAGCCAGAGGGCAGCCCCTGGAGCCGAAGGTCTACACCATGGGCCCTCCCCGG
GAGGAGCTGAGCAGCAGGTCGGTCAGCCTGACCTGCATGATCAACGGCTTCTACCCTTCCGACATCTCGGTGGAGTG
GGAGAAGAACGGGAAGGCAGAGGACAACTACAAGACCACGCCGGCCGTGCTGGACAGCGACGGCTCCTACTTCCTCT

ACAGCAAGCTCTCAGTGCCCACGAGTGAGTGGCAGCGGGGCGACGTCTTCACCTGCTCCGTGATGCACGAGGCCTTG
CACAACCACTACACGCAGAAGTCCATCTCCCGCTCTCCGGGTAAATGA
gcgctgtgccggcgagct*gcggccgc*

5

CLAIMS:

1. A method for depleting senescent cells endogenous to a subject, comprising administering to the subject a binding agent that is selectively toxic to senescent cells in an amount effective to reduce the number of such cells, wherein the binding agent binds selectively to a senescent cell surface protein having a misfolded conformation, relative to said protein in a native conformation.

2. The method according to claim 1, wherein the binding agent binds selectively to a misfolded form of human PrP.

3. The method according to claim 2, wherein the binding agent binds a misfolded human PrP epitope comprising a YYR motif.

4. The method according to claim 2, wherein the binding agent binds a misfolded human PrP epitope comprising a YML motif.

5. The method according to claim 2, wherein the binding agent binds a misfolded human PrP epitope comprising MDEYSNQNN (SEQ ID No.9).

6. The method according to claim 1, wherein the binding agent binds selectively to a misfolded form of human Fas receptor.

7. The method according to claim 6, wherein the binding agent binds a misfolded human Fas receptor epitope comprising LHHDGQFCH (SEQ ID No. 62).

8. The method according to claim 2, wherein the binding agent binds a misfolded human Fas receptor epitope comprising NSTVCEH (SEQ ID No. 65).

9. The method according to claim 1, wherein the binding agent binds selectively to a misfolded form of a surface protein selected from the human forms of Fas ligand, CD44, EGF receptor, CD38, Notch-1, CD44, CD59, and TNF receptor.

10. The method according to claims 1-9, wherein the binding agent is an antibody or a binding fragment thereof.

11. The method according to claim 10, wherein the antibody is a misfolded human PrP antibody that binds selectively to an epitope having the sequence of SEQ ID Nos. 1-14.

12. The method according to claim 11, wherein the misfolded human PrP antibody is the antibody 1A1 deposited with the International Depositary Authority of Canada under accession number 260210-01.

13. The method according to claim 11, wherein the misfolded human PrP antibody is an antibody comprising the following CDRs:

For the heavy chain:

CDR1	TYAMG	(SEQ ID No. 41)
CDR2	VITKSGNTYYASWAKG	(SEQ ID No. 42)
CDR3	YGIGVSYYDI	(SEQ ID No. 43)

For the light chain:

CDR1	QSSQSLYNKNWLS	(SEQ ID No. 44)
CDR2	KASTLES	(SEQ ID No. 45)
CDR3	QGEFSCSSADCTA	(SEQ ID No. 46).

14. The method according to claim 13, wherein the antibody is the antibody c-120.

15. The method according to claim 10, wherein the antibody is a misfolded human Fas receptor antibody that binds selectively to an epitope having the sequence of SEQ ID Nos. 62-66.

16 The method according to claims 1-15, for the treatment of an age-related degenerative disorder.

17. The method according to claim 16, for the treatment of cancer.

18. The method according to claim 16, for the treatment of emphysema.

19. The method according to claim 16, for the treatment of aging of the skin.

20. The method according to claim 16, for the treatment of sarcopenia.

21. The method according to claims 1-20, wherein the binding agent is a conjugate comprising an antibody or binding fragment thereof, and a second agent that is a toxin or a detectable label.

22. The method according to claim 21, wherein the second agent is a toxin.

23. A method for detecting senescent cells, comprising combining (1) a binding agent that binds selectively to a senescent cell surface protein having a misfolded conformation, and (2) a sample containing cells to be screened, and then determining the formation of a complex therebetween, the presence of such complex indicating that the sample contains a senescent cell.

24. The method according to claim 24, wherein the binding agent further comprises a detectable label.

25. The method according to claim 23, wherein the senescent state of the cell is confirmed by a second senescent cell detection method.

26. The method according to claim 25, wherein the second senescent cell detection method is a test for B-galactosidase activity at pH6.

27. A method for imaging senescent cells in a subject, comprising administering to the subject an imaging agent comprising a detectable label and, conjugated thereto, a binding agent that binds selectively to a senescent cell surface protein having a misfolded conformation, allowing the imaging agent to bind to any such senescent cells, and detecting the location of the detectable label in said subject.

28. A diagnostic kit comprising (1) an antibody that binds selectively to a senescent cell surface protein having a misfolded conformation, and (2) written instructions for the use thereof to detect senescent cells in accordance with the method defined in claim 23.

Fig. 1

HUVEC senescence staining with Alexa-488 (green fluorescence)

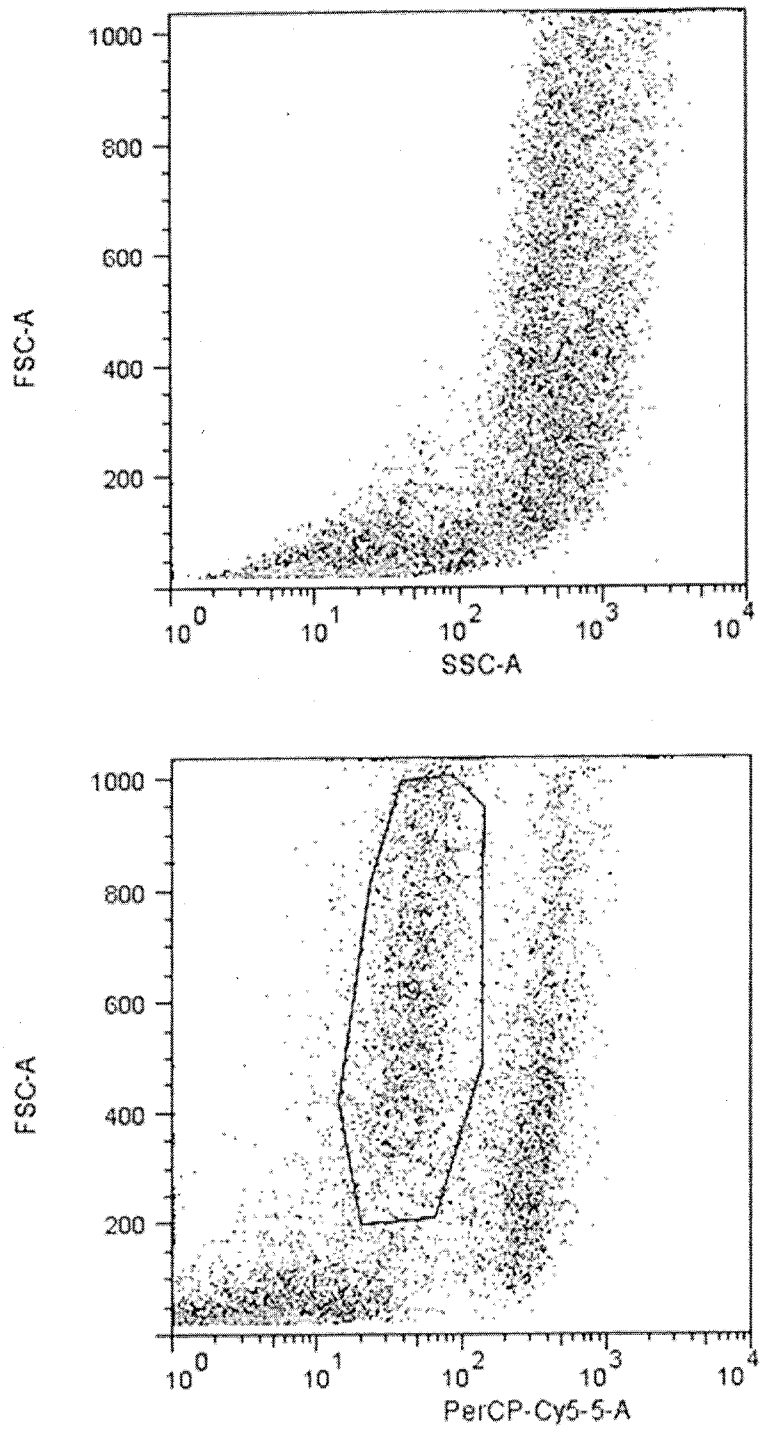


Fig. 1 Cont.

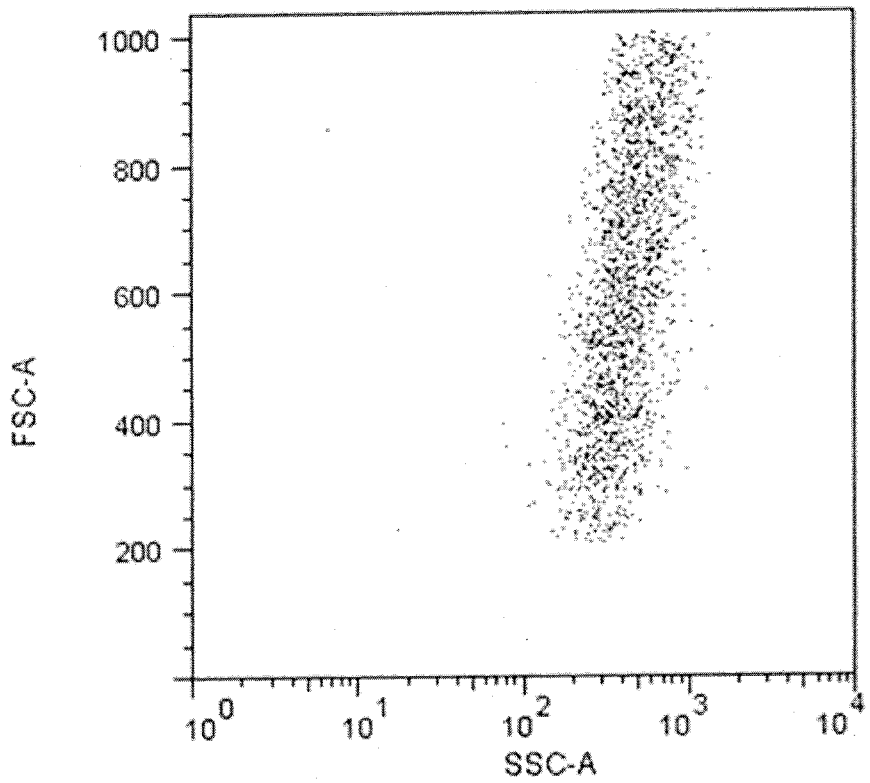


Fig. 2

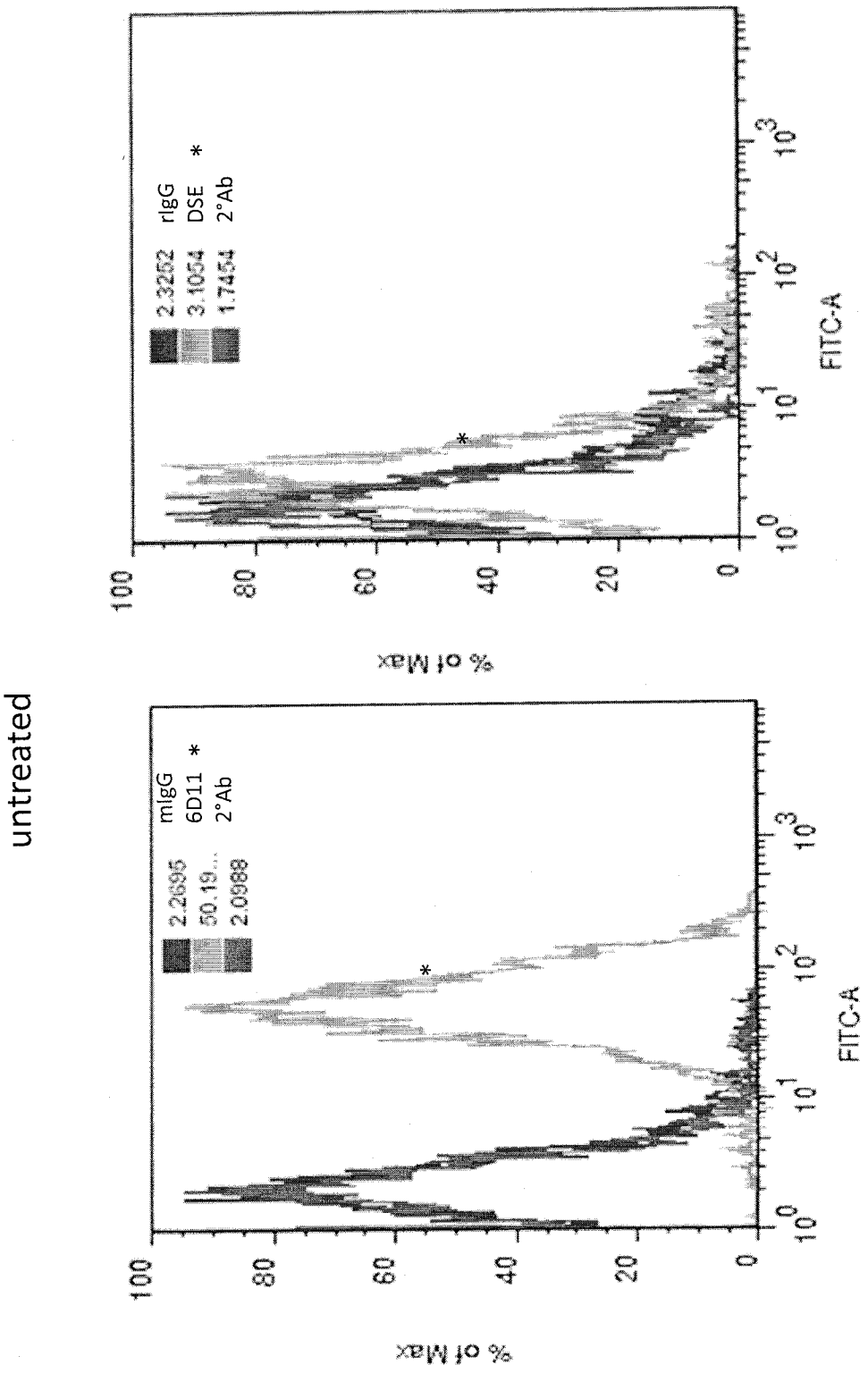

untreated
2.3252 rIgG
3.1054 DSE *
1.7454 2°Ab
2.2695 mIgG
50.19... 6D11 *
2.0988 2°Ab
FITC-A
% of Max
0
20
40
60
80
100
10^0
10^1
10^2
10^3

Fig. 2 Cont.

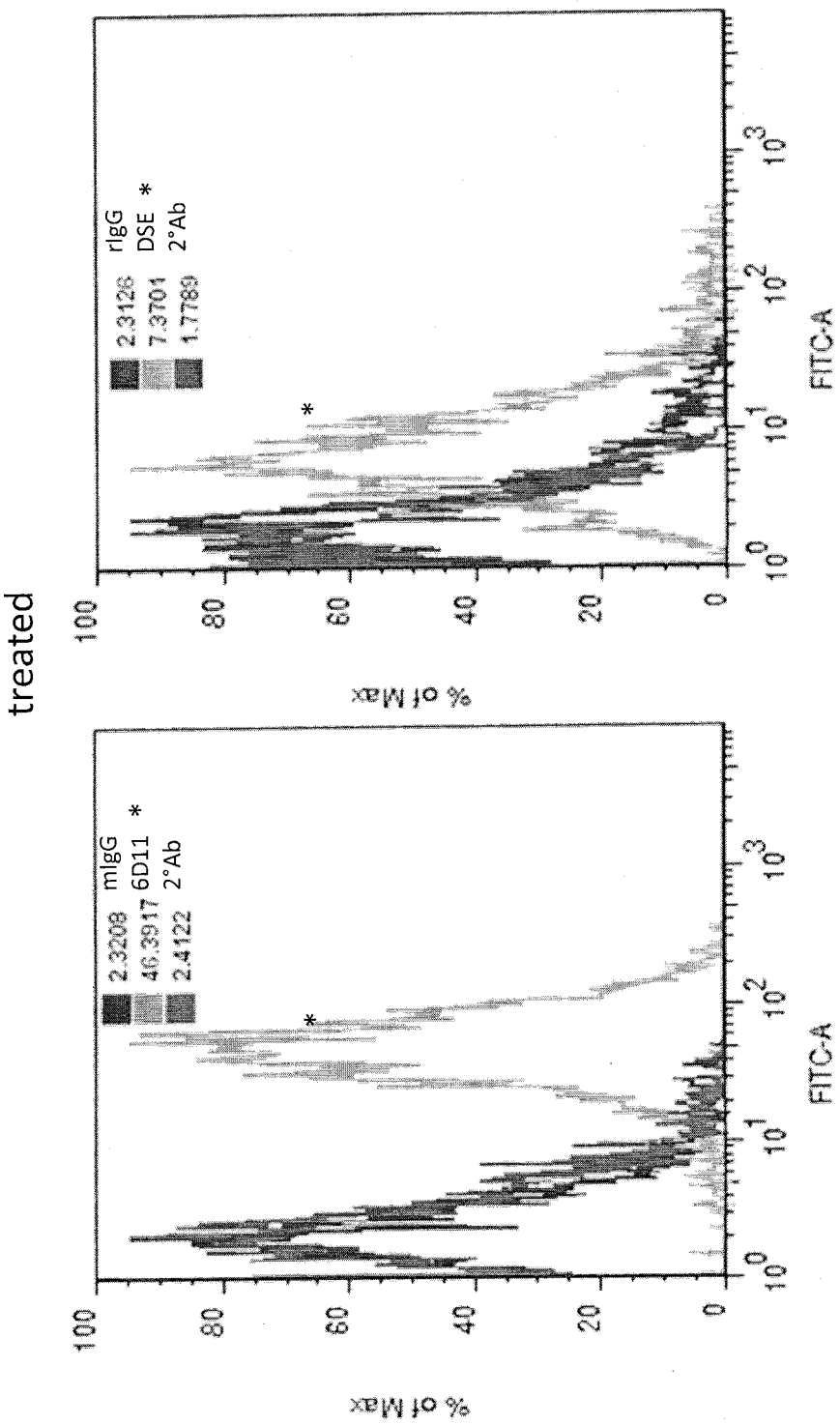

treated
rIgG
DSE *
2°Ab
2.3126
7.3701
1.7789
mIgG
6D11 *
2°Ab
2.3208
46.3917
2.4122
% of Max
FITC-A
0
20
40
60
80
100
10^0
10^1
10^2
10^3

Fig. 3

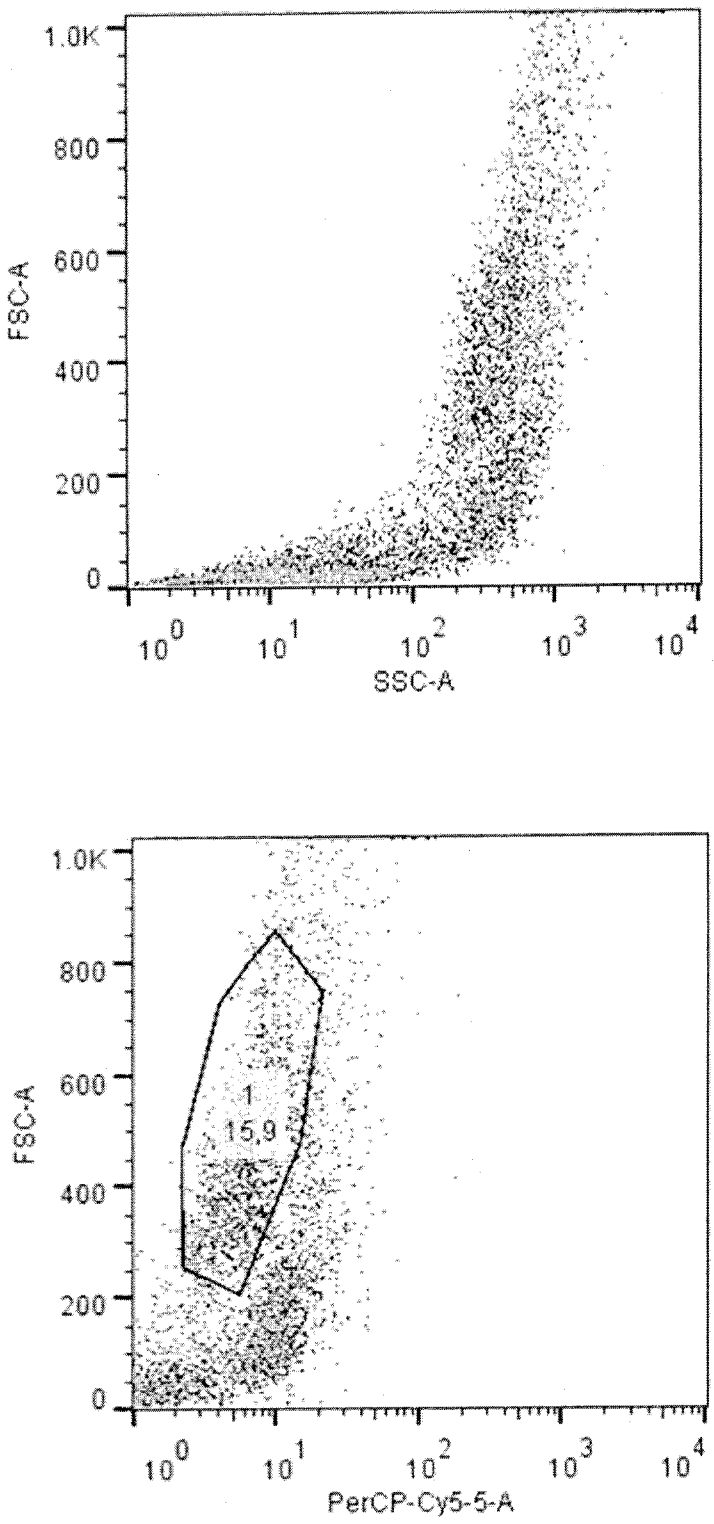
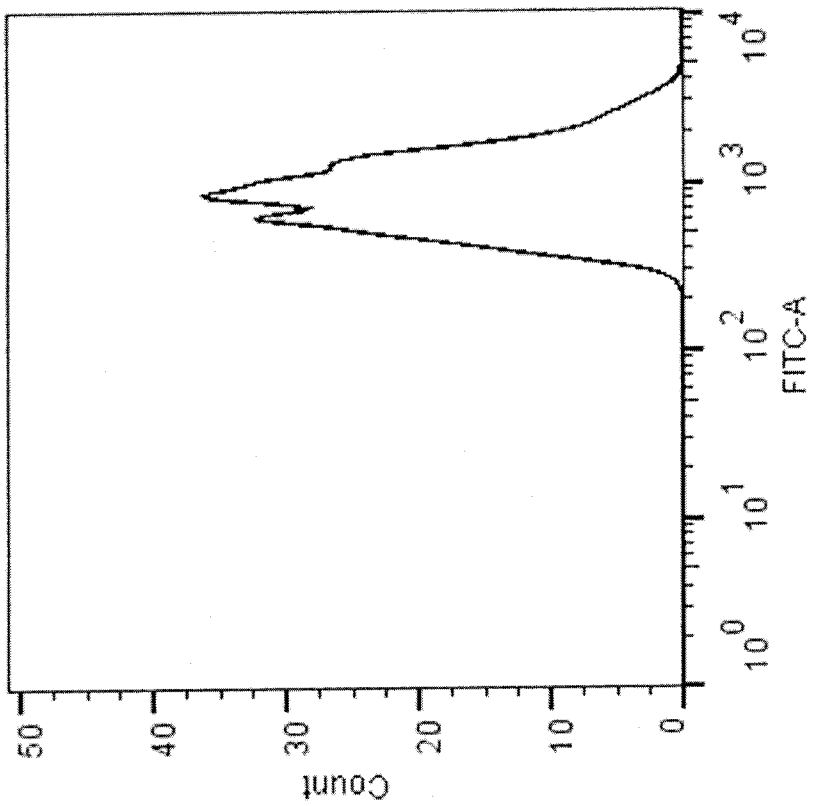

1.0K
800
600
400
200
0
FSC-A
10^0
10^1
10^2
10^3
10^4
SSC-A
1.0K
800
600
400
200
0
FSC-A
1
15,9
10^0
10^1
10^2
10^3
10^4
PerCP-Cy5-5-A

Fig. 3 Cont.



Senescence staining

Mitomycin c treated cells for 2 hrs.
Fas Abs: 50ug/ml
6D11: 10ug/ml

Fig. 4

A

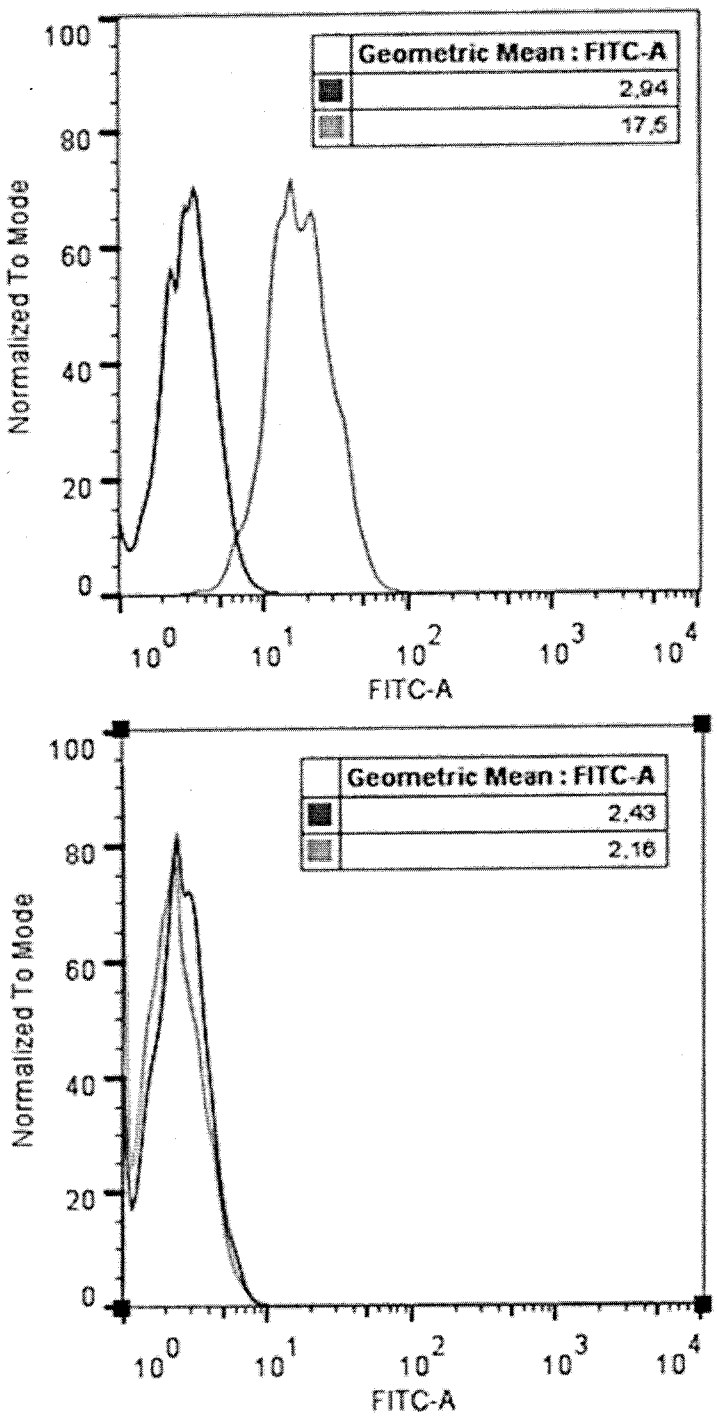

Geometric Mean : FITC-A
2.94
17.5
Normalized To Mode
100
80
60
40
20
0
10^0
10^1
10^2
10^3
10^4
FITC-A
Geometric Mean : FITC-A
2.43
2.16
Normalized To Mode
100
80
60
40
20
0
10^0
10^1
10^2
10^3
10^4
FITC-A

Fig. 4 Cont.

A

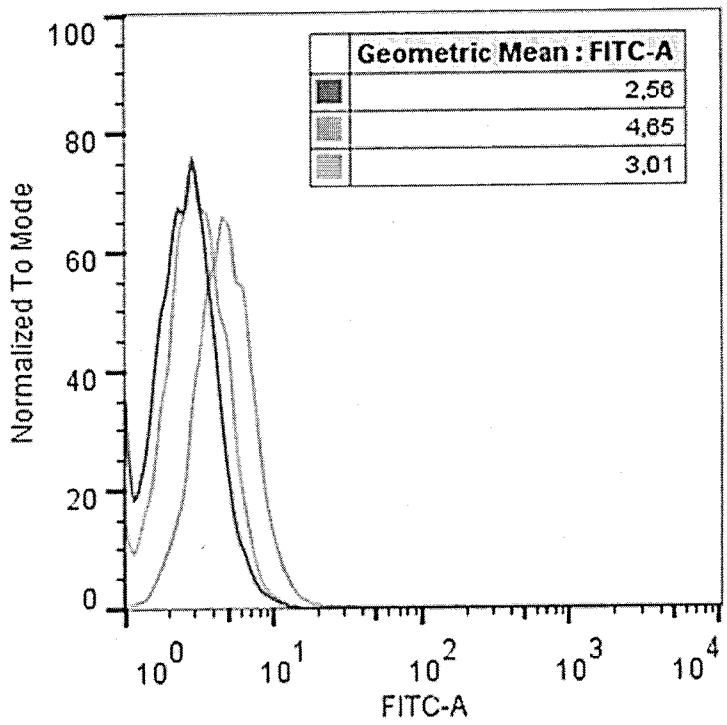

Geometric Mean : FITC-A
2,56
4,65
3,01
Normalized To Mode
100
80
60
40
20
0
10^0
10^1
10^2
10^3
10^4
FITC-A

Fig. 4 Cont.

B

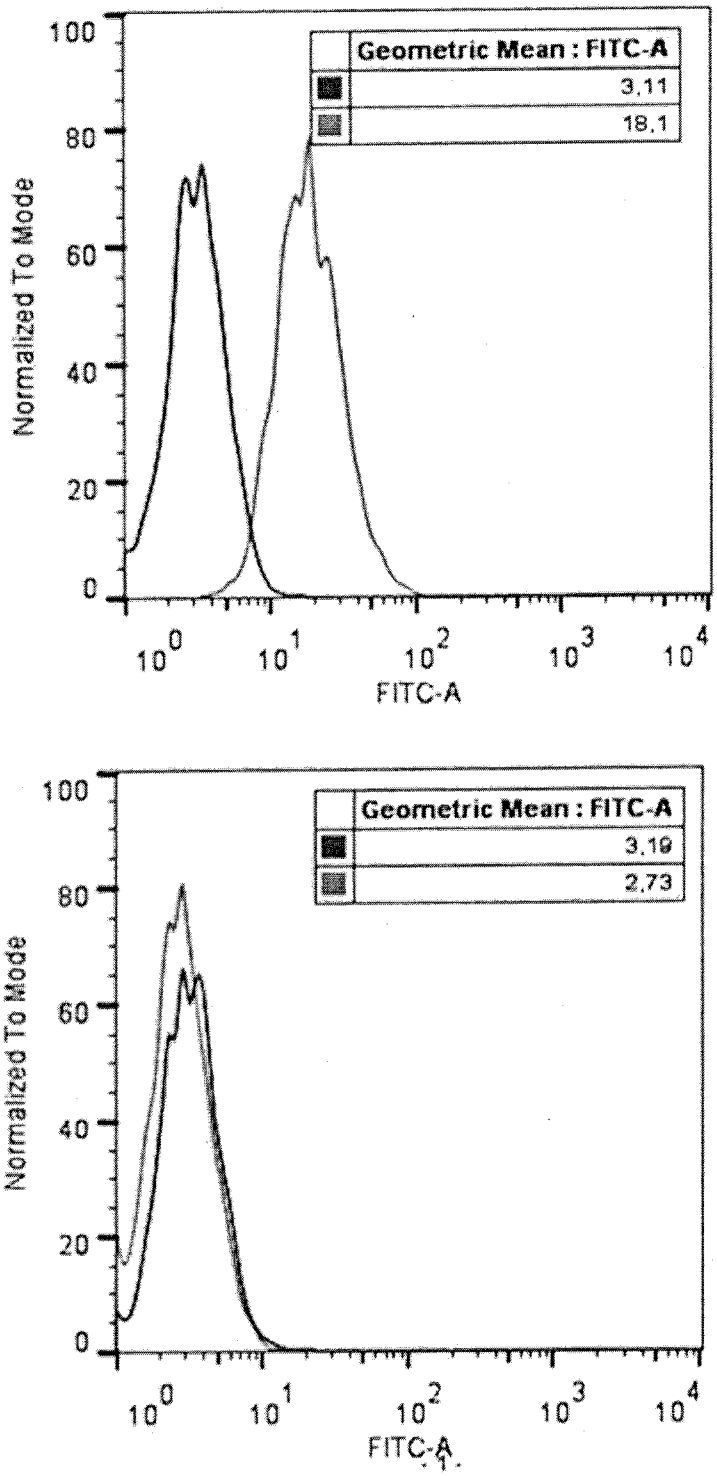
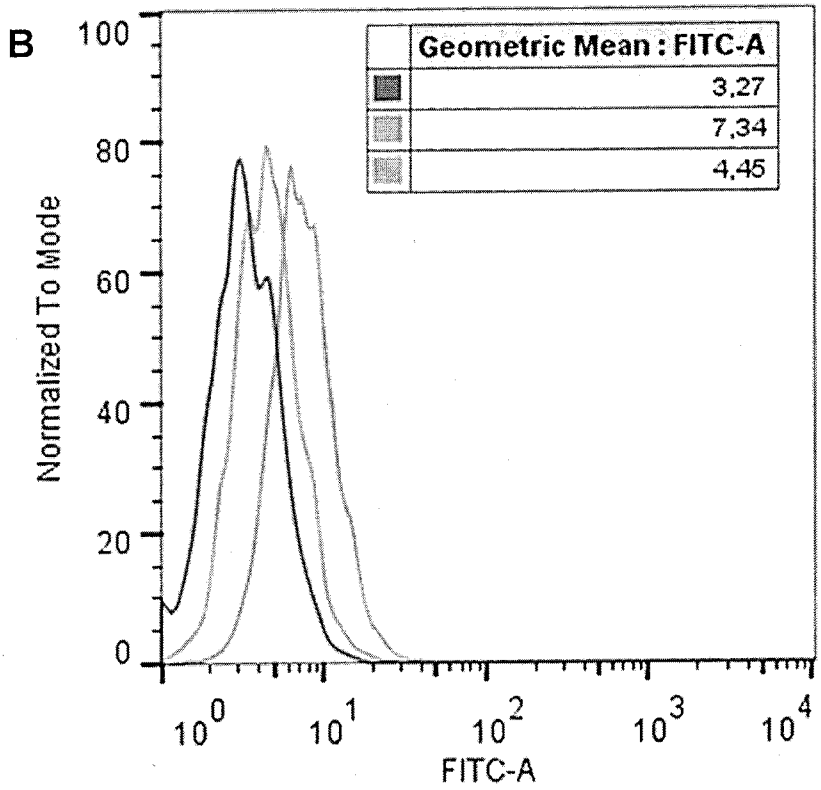

Geometric Mean : FITC-A
3,11
18,1
Normalized To Mode
100
80
60
40
20
0
10^0
10^1
10^2
10^3
10^4
FITC-A
Geometric Mean : FITC-A
3,19
2,73
Normalized To Mode
100
80
60
40
20
0
10^0
10^1
10^2
10^3
10^4
FITC-A

Fig. 4 Cont.


B
Geometric Mean : FITC-A
3,27
7,34
4,45
Normalized To Mode
100
80
60
40
20
0
10^0
10^1
10^2
10^3
10^4
FITC-A

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2014/000458

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***A61K 39/395*** (2006.01), ***A61K 47/48*** (2006.01), ***A61K 49/00*** (2006.01), ***A61P 25/00*** (2006.01), ***A61P 25/28*** (2006.01), ***A61P 35/00*** (2006.01), ***G01N 33/566*** (2006.01)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 39/395 (2006.01), A61K 47/48 (2006.01), A61K 49/00 (2006.01), A61P 25/00 (2006.01), A61P 25/28 (2006.01), A61P 35/00 (2006.01), G01N 33/566 (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
TotalPatent, Canadian Patent Database, Espacenet, Pubmed, Google Scholar, Keywords: senescent cell, misfolded protein, antibody, PrP, Fas receptor, Fas ligand, CD44, EGF receptor, CD38, Notch-1, CD59, TNF receptor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KAY, M. M. B., "Localization of senescent cell antigen on band 3", PNAS USA, September 1984 (09-1984), Vol. 81, pages 5753-5757, ISSN: 0027-8424, whole document	1, 10 and 16
Y		23-28
Y	PANI, G., "From growing to secreting: New roles for mTOR in aging cells", Cell Cycle, August 2011 (08-2011), Vol. 10, pages 2450-2453, ISSN: 1551-4005, whole document	23-28

☑ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier application or patent but published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 04 July 2014 (04-07-2014)	Date of mailing of the international search report 01 August 2014 (01-08-2014)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Authorized officer Yolanda Romsicki (819) 997-1044

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Golde, T. E and Miller, V. M, "Proteinopathy-induced neuronal senescence: a hypothesis for brain failure in Alzheimer's and other neurodegenerative diseases", Alzheimer's Research & Therapy, October 2009 (10-2009), Vol. 1, page 5, ISSN: 1758-9193, whole document	1-28

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claim Nos.: 1-22
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-22 encompass a method of medical treatment of a human or animal which this authority is not obliged to search under Rule 39.1 of the PCT, the search has been carried out based on the alleged use of the binding agent referred to therein for depleting senescent cells endogenous to a subject. Although claims 23-27 are not considered to encompass a method of medical treatment at this time, these claims could be considered to encompass a method of medical treatment in certain jurisdictions.

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

The claims directed to a plurality of inventive concepts as follows:

Group A - Claims 1, 10 and 16-21 (all partially), 2-5 (all entirely) and 11-14 (all entirely) are directed towards a method for depleting senescent cells endogenous to a subject using a binding agent that selectively binds to a misfolded form of human PrP;

Continued on extra sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☑ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant=s protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Continuation of Box No. III

Group B - Claims 1, 10 and 16-21 (all partially) and 6-8 (all entirely) are directed towards a method for depleting senescent cells endogenous to a subject using a binding agent that selectively binds to a misfolded form of human Fas receptor;

Group C - Claims 1, 9, 10 and 16-21 (all partially) are directed towards a method for depleting senescent cells endogenous to a subject using a binding agent that selectively binds to a misfolded form of human Fas ligand;

Group D - Claims 1, 9, 10 and 16-21 (all partially) are directed towards a method for depleting senescent cells endogenous to a subject using a binding agent that selectively binds to a misfolded form of human CD44;

Group E - Claims 1, 9, 10 and 16-21 (all partially) are directed towards a method for depleting senescent cells endogenous to a subject using a binding agent that selectively binds to a misfolded form of human EGF receptor;

Group F - Claims 1, 9, 10 and 16-21 (all partially) are directed towards a method for depleting senescent cells endogenous to a subject using a binding agent that selectively binds to a misfolded form of human CD38;

Group G - Claims 1, 9, 10 and 16-21 (all partially) are directed towards a method for depleting senescent cells endogenous to a subject using a binding agent that selectively binds to a misfolded form of human Notch-1;

Group H - Claims 1, 9, 10 and 16-21 (all partially) are directed towards a method for depleting senescent cells endogenous to a subject using a binding agent that selectively binds to a misfolded form of human CD59;

Group I - Claims 1, 9, 10 and 16-21 (all partially) are directed towards a method for depleting senescent cells endogenous to a subject using a binding agent that selectively binds to a misfolded form of human TNF receptor; and

Group J - Claims 23-28 (all entirely) are directed towards a method for detecting or imagine senescent cells in a subject using a binding agent that binds selectively to a senescent cell surface protein, and a diagnostic kit relating to same.

Since the claims are directed towards methods involving binding agents that selectively bind to distinct cell surface proteins having misfolded conformations, claims 1-28 are not linked by a single inventive concept.