

1 **"Therapy"**

2

3 **Field of the Invention**

4

5 This application relates to methods of treatment of
6 neoplastic disease and compositions for use in such
7 treatments. In particular, it relates to antibody
8 based therapies for the treatment of cancer as well
9 as combination therapies to counteract tumour cell
10 defence mechanisms to chemotherapeutic treatments.

11

12 **Background to the Invention**

13

14 Proteases are a large group of proteins that
15 comprise approximately 2% of all gene products
16 (Rawlings and Barrett, 1999). Proteases catalyse the
17 hydrolysis of peptide bonds and are vital for the
18 proper functioning of all cells and organisms.
19 Proteolytic processing events are important in a
20 wide range of cellular processes including bone
21 formation, wound healing, angiogenesis and
22 apoptosis.

23

24 The lysosomal cysteine proteases were initially
25 thought to be enzymes that were responsible for non-
26 selective degradation of proteins in the lysosomes.
27 They are now known to be accountable for a number of
28 important cellular processes, having roles in
29 apoptosis, antigen presentation, coagulation,
30 digestion, pro-hormone processing and extracellular
31 matrix remodelling (Chapman *et al*, 1997).

32

1 Cathepsin S (Cat S) is a member of the papain
2 superfamily of lysosomal cysteine proteases. To
3 date, eleven human cathepsins have been identified,
4 but the specific *in vivo* roles of each are still to
5 be determined (Katunuma *et al*, 2003). Cathepsins B,
6 L, H, F, O, X and C are expressed in most cells,
7 suggesting a possible role in regulating protein
8 turnover, whereas Cathepsins S, K, W and V are
9 restricted to particular cells and tissues,
10 indicating that they may have more specific roles
11 (Kos *et al*, 2001; Berdowska, 2004, *Clinica Chimica*
12 *Acta*. 2004; 342: 41-69).
13
14 Cat S was originally identified from bovine lymph
15 nodes and spleen and the human form cloned from a
16 human macrophage cDNA library (Shi *et al*, *J Biol*
17 *Chem*. 1992; 267: 7258-7262). The gene encoding Cat S
18 is located on human chromosome 1q21. The 996 base
19 pair transcript encoded by the Cat S gene is
20 initially translated into an unprocessed precursor
21 protein with a molecular weight of 37.5 kDa. The
22 unprocessed protein is composed of 331 amino acids;
23 a 15 amino acid signal peptide, a 99 amino acid pro-
24 peptide sequence and a 217 amino acid peptide. Cat S
25 is initially expressed with a signal peptide that is
26 removed after it enters the lumen of the endoplasmic
27 reticulum. The propeptide sequence binds to the
28 active site of the protease, rendering it inactive
29 until it has been transported to the acidic
30 endosomal compartments, after which the propeptide
31 sequence is removed and the protease is activated

1 (Baker *et al*, 2003 *Protein Expr Purif*. 28,
2 93-101).
3
4 Cat S has been identified as a key enzyme in major
5 histocompatibility complex class II (MHC-II)
6 mediated antigen presentation, by cleavage of the
7 invariant chain, prior to antigen loading. Studies
8 have shown that mice deficient in Cat S have an
9 impaired ability to present exogenous proteins by
10 APCs (Nakagawa *et al*, *Immunity*. 1999; 10: 207-217).
11 The specificity of Cat S in the processing of the
12 invariant chain Ii, allows for Cat S specific
13 therapeutic targets in the treatment of conditions
14 such as asthma and autoimmune disorders (Chapman *et*
15 *al*, 1997).
16

17 Pathological association of Cat S

18
19 Alterations in protease control frequently underlie
20 many human pathological processes. The deregulated
21 expression and activity of the lysosomal cysteine
22 protease Cathepsin S has been linked to a range of
23 conditions including neurodegenerative disorders,
24 autoimmune diseases and certain malignancies.
25

26 Cat S upregulation has been linked to several
27 neurodegenerative disorders. It is believed to have
28 a role in the production of the β peptide (A β) from
29 the amyloid precursor protein (APP) (Munger *et al*,
30 ***Biochem. J.* 1995; 311: 299-305**) and its expression
31 has been shown to be upregulated in both Alzheimer's
32 Disease and Down's Syndrome (Lemere *et al*, 1995).

1 Cat S may also have a role in Multiple Sclerosis
2 through the ability of Cat S to degrade myelin basic
3 protein, a potential autoantigen implicated in the
4 pathogenesis of MS (Beck et al, 2001, **Eur. J.**
5 **Immunol.** 2001; 31: 3726-3736) and in Creutzfeldt -
6 Jakob disease (CJD) patients, Cat S expression has
7 been shown to increase more than four fold (Baker et
8 al, 2002).

9
10 Cathepsin S has been reported to be overexpressed in
11 atherosclerotic and restenosis after angioplasty
12 (Cheng et al, Am J Pathology, 2006, 168:685-694). In
13 these conditions, the CatS was reported to co-
14 localise with integrin $\alpha\beta 3$ as a receptor on the
15 vascular smooth muscle cell surface.

16
17 Angiogenesis, the development of microvasculature,
18 is an integral process within many normal
19 physiological processes such as normal development
20 and wound healing. Angiogenesis is characterised by
21 the stimulation of endothelial cells to form primary
22 blood vessels where a non-clarified complex
23 interplay exists between the endothelial cells,
24 surrounding microenvironment and a range of pro- and
25 anti-angiogenic factors. However, uncontrolled or
26 inappropriate angiogenesis is accepted as an
27 underlying factor to the pathology of a wide range
28 of diseases including tumour progression and ocular
29 disease.

30
31 The association of CatS with angiogenesis was first
32 shown *in vitro* using CatS deficient endothelial

1 cells (Shi et al, 2003). Microvascular endothelial
2 cells (ECs) have been shown to secrete proteases,
3 permitting penetration of the vascular basement
4 membrane as well as the interstitial extracellular
5 matrix. Treatment of cultured ECs with inflammatory
6 cytokines or angiogenic factors stimulated
7 expression of CatS, and its inhibition reduced
8 microtubule formation. CatS -/- mice displayed
9 defective microvessel development during wound
10 repair in comparison to wild-type controls (Shi et
11 al, 2003).

12
13 Further examination of the role of CatS in
14 angiogenesis and tumour growth was demonstrated in a
15 transgenic mouse model for pancreatic islet cell
16 carcinoma. CatS-/- mice were found to develop
17 significantly smaller tumours and fewer angiogenic
18 islets in comparison to the CatS+/+ control mice
19 (Gocheva et al., 2006). Insight to the molecular
20 mechanism underpinning this phenotype was
21 subsequently provided by evidence that CatS could
22 cleave and inactivate anti-angiogenic peptides and
23 promote the generation of active pro-angiogenic
24 fragments (Wang et al, 2006).

25
26 The role of Cat S has also been investigated in
27 specific malignancies. The expression of Cat S was
28 shown to be significantly greater in lung tumour and
29 prostate carcinomas sections in comparison to normal
30 tissue (Kos et al, 2001, Fernandez et al, 2001) and
31 suggested that Cat S may have a role in tumour
32 invasion and disease progression.

1
2 Recent work on Cat S demonstrated the significance
3 of its expression in human astrocytomas (Flannery *et*
4 *al*, 2003). Immunohistochemical analysis showed the
5 expression of Cat S in a panel of astrocytoma biopsy
6 specimens from WHO grades I to IV, but appeared
7 absent from normal astrocytes, neurones,
8 oligodendrocytes and endothelial cells. Cat S
9 expression appeared highest in grade IV tumours and
10 levels of extracellular activity were greatest in
11 cultures derived from grade IV tumours.

12
13 Cat S has been shown to be active in the degradation
14 of ECM macromolecules such as laminin, collagens,
15 elastin and chondroitin sulphate proteoglycans
16 (Liuzzo *et al*, 1999). Using invasion assays with
17 the U251MG grade IV glioblastoma cell line, a 61%
18 reduction in invasion in the presence of a Cat S
19 inhibitor LHVS29 has been shown (Flannery *et al*,
20 2003).

21
22 The generation of inhibitors specifically targeting
23 Cat S have potential as therapeutic agents for
24 alleviations of the symptoms associated with the
25 activity of this protease.

26
27 The implication of aberrant extracellular cysteine
28 cathepsin activity in tumour progression has been of
29 particular focus to researchers. Each of these
30 lysosomal enzymes has been implicated in the
31 progression of various tumours, where it is thought
32 that their abnormally high secretion from tumour

1 cells leads to the degradation of the extracellular
2 matrix (ECM). This aberrant breakdown of ECM
3 components such as elastin and collagen accelerates
4 the penetration and invasion of these abnormal cells
5 to surrounding normal tissue. Additionally, roles
6 in angiogenesis and the processing of other
7 molecules have also been attributed to inappropriate
8 cathepsin activity (Lah and Kos, 1998 Biol Chem..
9 379, 125-30; Folkman and Ingber, 1992; Fernandez et
10 al, 2001).

11
12 Much research has focussed on the underlying
13 mechanisms that result in this devastating increase
14 in extracellular proteolytic activity. Cathepsins
15 are believed to be involved in the degradation of
16 the ECM directly through their ability to degrade
17 components of the ECM such as laminin, fibronectin
18 and collagen or indirectly through the activation of
19 other proteases in a proteolytic cascade (Koblinski
20 et al, 2000; Rao et al, 2003).

21 22 Inhibition of Cat S

23
24 When proteases are over-expressed, therapeutic
25 strategies have focused on the development of
26 inhibitors to block the activity of these enzymes.
27 The generation of specific small molecule inhibitors
28 to the cathepsins have proved difficult in the past,
29 due to problems with selectivity and specificity.
30 The dipeptide α -keto- β -aldehydes developed as potent
31 reversible inhibitors to Cat S by Walker et al, had
32 the ability to inhibit Cat B and L, albeit with less

1 efficiency (Walker *et al*, *Biochem. Biophys. Res.*
2 *Comm.* 2000; 75: 401-405), and the Cat S inhibitor 4-
3 Morpholineurea-Leu-HomoPhe-vinylsulphone (LHVS) has
4 also been shown to inhibit other cathepsins when
5 used at higher concentrations (Palmer *et al*, *J. Med.*
6 *Chem.* 1995; 38: 3193-3196).

7
8 A broad range of antineoplastics have been developed
9 for the treatment of cancer. However, although many
10 of these compounds are successfully used in
11 treatment strategies, in many cases a particular
12 treatment regime does not result in complete
13 clearance of neoplasms or only does so temporarily.
14 There thus remains a great need for the development
15 of new cancer treatments and therapeutic regimes.

16

17 **Summary of the Invention**

18

19 As described herein, the present inventors have
20 unexpectedly found that Cathepsin S (CatS) can be
21 found on the cell surface of tumour cell lines. When
22 a CatS monoclonal antibody was employed as an
23 isotype control in an unrelated tumour treatment
24 study the inventors discovered positive staining of
25 tumour cells with the antibody.

26

27 The surprising discovery by the present inventors
28 that, contrary to the commonly held belief in the
29 art that this molecule was localised internally in
30 lysosomes in tumour cells or secreted from such
31 cells, Cathepsin S is localised on the cell surface

1 of tumour cells, provides a means to identify tumour
2 cells in a population of cells.

3

4 Accordingly, in a first aspect of the present
5 invention, there is provided a method of identifying
6 a tumour cell in a population of cells, said method
7 comprising bringing into contact a ligand with
8 binding specificity for Cathepsin S with said
9 population of cells and determining the presence or
10 absence of binding of the ligand to cells in said
11 population, wherein binding is indicative of the
12 presence of tumour cells within said population of
13 cells.

14

15 The method of the first aspect of the invention may
16 be performed in vitro. In another embodiment, the
17 method is performed in vivo.

18

19 The invention also enables the cell-surface CatS to
20 be employed as a biomarker for the use of antibody
21 delivered drug conjugates. This strategy could be
22 further exploited by the delivery of pro-drugs to
23 the cells, which are activated specifically by the
24 enzymatic activity of CatS.

25

26 In a second aspect of the present invention, there
27 is provided the use of Cathepsin S as a biomarker
28 for tumour cells.

29

30 Moreover, the present inventors have found that
31 treatment of tumour derived cell lines with
32 chemotherapeutic agents leads to enhanced cell

1 surface localisation of Cathepsin S. This implies
2 that the chemotherapy could therefore counter-
3 productively promote the degradation of the ECM,
4 increasing the invasive properties of the aberrant
5 cells. This effect could have severe implications
6 for the prognosis of the patient being treated with
7 such chemotherapy.

8
9 However, the efficacy of existing chemotherapeutic
10 agents could be improved by the targeting of this
11 cell surface CatS. Inhibitors to CatS, for example
12 small molecule, peptide/protein, or antibodies,
13 could be used in combination with chemotherapeutic
14 agents to ensure that the chemotherapy does not
15 result in the generation of a sub-population of
16 cells that are hyper-invasive.

17
18 Thus, in a third aspect of the present invention,
19 there is provided a method of inhibiting
20 chemotherapy induced upregulation of Cathepsin S on
21 the surface of tumour cells, said method comprising
22 the administration of a Cathepsin S inhibitor to
23 said cells.

24
25 In a fourth aspect of the invention there is
26 provided a method of treating neoplastic disease
27 comprising the simultaneous, sequential or separate
28 administration of a Cathepsin S inhibitor and a
29 chemotherapeutic agent.

30
31 In a fifth aspect, the invention provides the use of
32 a Cathepsin S inhibitor and a chemotherapeutic agent

1 in the preparation of a medicament for combination
2 therapy by simultaneous, sequential or separate
3 administration of the Cathepsin S inhibitor and
4 chemotherapeutic agent in the treatment of a
5 neoplastic disease.

6

7 In a sixth aspect of the invention, there is
8 provided a pharmaceutical composition comprising a
9 Cathepsin S inhibitor and a chemotherapeutic agent.

10

11 In one embodiment of the invention the Cathepsin S
12 inhibitor and chemotherapeutic agent are provided in
13 concentrations which produce a synergistic effect.

14

15 As described in the Examples, the upregulation of
16 Cathepsin S by chemotherapeutic agents was not
17 limited to one class of antineoplastic but appears
18 to be common to many or all classes. The method of
19 the invention may therefore find use in treatment
20 regimes involving any class of chemotherapeutic
21 agent. For example, chemotherapeutic agents, which
22 may be used in the present invention include
23 platinum based antineoplastics, antimetabolites,
24 nucleoside analogs, thymidylate synthase inhibitors,
25 ortopoisomerase inhibitors.

26

27 In one embodiment, the chemotherapeutic agent is a
28 platinum based antineoplastic, for example cisplatin
29 or oxaliplatin.

30

1 In another particular embodiment, the
2 chemotherapeutic agent is a thymidylate synthase
3 inhibitor, for example 5-FU.

4

5 In another particular embodiment, the
6 chemotherapeutic agent is a topoisomerase inhibitor,
7 for example CPT-11.

8

9 Any suitable Cathepsin S inhibitor may be used in
10 the present invention. Such inhibitors may be, for
11 example, small molecule inhibitors, peptides or
12 antibodies, or nucleic acid molecules encoding said
13 peptides or antibodies. In one embodiment, the
14 Cathepsin S inhibitor is a small molecule inhibitor
15 such as a dipeptide α -keto- β -aldehyde or 4-
16 Morpholineurea-Leu-HomoPhe-vinylsulphone (LHVS). In
17 another embodiment, the Cathepsin S inhibitor is an
18 antibody molecule, such as an antibody or antibody
19 fragment.

20

21 As detailed in the applicant's co pending
22 international patent application which claims
23 priority from GB0507219.4 and GB0507272.3,
24 WO2006/109045, the present inventors have identified
25 a novel class of Cathepsin S antibodies, the
26 antibodies having potent antiproteolytic activity.
27 Moreover, the antibodies are shown to have
28 significant inhibitory effects on tumour invasion
29 and angiogenesis. The class is exemplified by a
30 monoclonal antibody 1E11 for which the inventors
31 have identified the VH and VL domains and CDRs of
32 the antibody. This is the first demonstration of a

1 Cathepsin S specific antibody directly inhibiting
2 the protease activity of Cathepsin S and thus
3 uniquely enables the use of such antibodies as
4 active therapeutic agents with a wide range of
5 applications from cancer therapeutics to anti-
6 inflammatory agents with high specificity and low
7 toxicity.

8
9 Accordingly, in one embodiment of the present
10 invention, the Cathepsin S inhibitor is an antibody
11 molecule, which binds Cathepsin S and inhibits its
12 proteolytic activity. Any antibody molecule capable
13 of inhibiting the proteolytic effect of Cathepsin S
14 may be used in such aspects of the invention.

15
16 In an embodiment of the invention, the antibody
17 molecule comprises an antigen binding domain
18 comprising at least one of the CDRs with an amino
19 acid sequence selected from the group consisting of
20 Seq ID No: 1, Seq ID No: 2, Seq ID No: 3, and/or at
21 least one of the CDRs with an amino acid sequence
22 consisting of Seq ID No: 4, Seq ID No: 5 and Seq ID
23 No: 6, .

24
25 The amino acid sequences corresponding to SEQ ID
26 NOS: 1-6 are as follows:

27
28 Seq ID No: 1:

29 SYDMS

30

31 Seq ID No: 2:

32 YITTGGVNTYYPDTVKG

1

2 Seq ID No: 3

3 HSYFDY

4

5 Seq ID No: 4:

6 RSSQSLVHSNGNTYLH

7

8 Seq ID No: 5:

9 KVSNRFS

10

11 Seq ID No: 6:

12 SQTTHVPPT

13

14

15 In an embodiment, the antibody molecule comprises an
16 antigen binding domain comprising at least one, for
17 example at least two or all three of the CDRs with
18 an amino acid sequence selected from the group
19 consisting of Seq ID No: 1, Seq ID No: 2, and Seq ID
20 No: 3, or variants thereof and at least one, for
21 example at least two, for example all three of the
22 CDRs with an amino acid sequence selected from the
23 group consisting of Seq ID No: 4, Seq ID No: 5 and
24 Seq ID No: 6., or variants thereof

25

26 In another embodiment, the antibody molecule
27 comprises an antigen binding domain comprising at
28 least one of the CDRs with an amino acid sequence
29 selected from the group consisting of Seq ID No: 1,
30 Seq ID No: 2, and Seq ID No: 3, and/or at least one
31 of the CDRs with an amino acid sequence selected

1 from the group consisting of Seq ID No: 4, Seq ID
2 No: 5 and Seq ID No: 6.

3
4 In a particular embodiment, the antibody molecule
5 comprises a CDR having the amino acid sequence SEQ
6 ID NO: 5, or a variant thereof and/or the CDR having
7 the amino acid sequence SEQ ID NO: 6, or a variant
8 thereof.

9
10 In one embodiment, the antibody molecule comprises
11 an antibody V_H domain or an antibody V_L domain, or
12 both.

13
14 In one embodiment, the antibody molecule has an
15 antibody V_H domain which comprises at least one of
16 the CDRs, for example two or three CDRs, with an
17 amino acid sequence selected from the group
18 consisting of Seq ID No: 1, Seq ID No: 2, Seq ID No:
19 3, and/or the antibody V_L domain comprises at least
20 one of the CDRs, for example two or three CDRs, with
21 an amino acid sequence consisting of Seq ID No: 4,
22 Seq ID No: 5 and Seq ID No: 6.

23
24 In a preferred embodiment, the antibody V_L domain
25 comprises the amino acid sequence Seq ID No: 8 and
26 /or the antibody V_H domain comprises the amino acid
27 sequence Seq ID No: 7.

28

29 Seq ID No: 7:

30 VQLQESGGVLVKPGGSLKLSCAASGFAFSSYDMSWVRQTPEKRLEWVAYITT
31 GGVNTYYPDITVKGRFTISRDNKNTLYLQMSSLKSEDTAMYYCARHSYFDY
32 WGQGTITVTVSS

33

1 Seq ID No: 8:

2 DVLMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWYLQKPGQSPKLL
3 IYKVSNRFSGVDPDRFSGSGGTDFTLKISRVEAEDLG VYFCSQTTHVPPTFG
4 SGTKELEIKR

5
6

7 The antibody molecule may be an antibody, for
8 example a whole antibody.

9

10 In one alternative embodiment, the antibody molecule
11 may be an antibody fragment such as a scFv.

12

13 Further antibody molecules which may be used in the
14 present invention include antibody molecules
15 comprising at least one, for example one, two or
16 three, of the CDRs with an amino acid sequence
17 selected from the group consisting of Seq ID No: 1,
18 Seq ID No: 2, Seq ID No: 3, and/or at least one, for
19 example one, two or three, of the CDRs with an amino
20 acid sequence consisting of Seq ID No: 4, Seq ID No:
21 5 and Seq ID No: 6, in which 5 or less, for example
22 4, 3, 2, or 1 amino acid substitutions have been
23 made in at least one CDR and wherein the antibody
24 molecule retains the ability to inhibit the
25 proteolytic activity of Cathepsin S.

26

27 In an embodiment of the invention, the antibody
28 molecule for use in the invention has the ability to
29 inhibit tumour cell invasion.

30

31 In another embodiment, the antibody molecule for
32 use in the invention has the ability to inhibit
33 angiogenesis.

1

2 As described above, the present inventors have
3 surprisingly shown that, in tumour cells, Cathepsin
4 S is localised on the cell surface and, moreover, in
5 tumour cells subjected to chemotherapeutic agents,
6 Cathepsin S expression on the surface of the tumour
7 cells, is upregulated. This demonstration, as well
8 as enabling the identification of tumour cells using
9 antibodies against Cathepsin S and the use of
10 combination therapies of a Cathepsin S inhibitor and
11 a chemotherapeutic agent, also suggests that anti-
12 Cathepsin S antibodies may be used in the absence of
13 chemotherapeutic agents to induce ADCC in tumour
14 cells.

15

16 Accordingly, in a seventh aspect of the present
17 invention, there is provided a method of inducing
18 antibody dependent cell-mediated cytotoxic (ADCC)
19 reaction against a tumour cell, said method
20 comprising the administration of an anti-Cathepsin S
21 antibody molecule which binds Cathepsin S to said
22 cell.

23

24 By inducing ADCC, tumour cells may be killed. Thus,
25 in an eighth aspect of the present invention, there
26 is provided a method of killing tumour cells, said
27 method comprising the administration of an anti-
28 Cathepsin S antibody molecule which binds Cathepsin
29 S to said cells.

30

1 The methods of the seventh and eighth aspects of the
2 invention may be performed in vitro, in vivo or ex
3 vivo, as required.

4

5 Accordingly, in a ninth aspect of the present
6 invention, there is provided a method of treating
7 neoplastic disease in a subject, said method
8 comprising the administration of an anti-Cathepsin S
9 antibody molecule which binds Cathepsin S to said
10 subject.

11

12 In an tenth aspect, the invention provides the use
13 of an anti-Cathepsin S antibody molecule which binds
14 Cathepsin S in the preparation of a medicament for
15 the treatment of a neoplastic disease.

16

17 Moreover, the demonstration that Cathepsin S is
18 localised on the surface of tumour cells suggests
19 that, to induce an anti-tumour cell effect, there is
20 no requirement that the antibodies which bind
21 Cathepsin S need have any anti-proteolytic activity.

22

23 Thus, in one embodiment of any one of the seventh to
24 tenth aspects of the present invention, the antibody
25 molecule thereof does not inhibit the proteolytic
26 activity of Cathepsin S.

27

28 In one embodiment of any one of the seventh to tenth
29 aspects of the present invention, the anti-Cathepsin
30 S antibody molecule is administered in the absence
31 of another chemotherapeutic agent, i.e. in contrast

1 to the methods of any one of the first to sixth
2 aspects of the present invention.

3

4 As described above, in the methods of the invention,
5 anti Cathepsin S antibody molecules may be used
6 which do not inhibit the proteolytic effect of
7 Cathepsin S. The therapeutic use of such antibody
8 molecules is further supported by the demonstration
9 by the present inventors, as described in the
10 Examples, of the anti-angiogenic effects of such
11 anti - Cathepsin S antibody molecules.

12

13 Specifically, the present inventors have shown for
14 the first time that antibodies, which have
15 specificity for Cathepsin S, but which do not
16 inhibit the proteolytic activity of Cathepsin S,
17 nevertheless act as potent inhibitors of
18 angiogenesis. This was particularly surprising to
19 the inventors given that, as described in
20 PCT/GB2006/001314, it was believed that, to
21 counteract the pathological effects of dysregulation
22 of Cathepsin S activity, strategies are required
23 which inhibit the proteolytic effects of Cathepsin
24 S. Furthermore, although the use of antibodies to
25 sequester proteins has been suggested as a
26 therapeutic strategy for some proteins, e.g. VEGF,
27 such a strategy has not, until now, been considered
28 as feasible for the treatment of diseases associated
29 with Cathepsin S activity.

30

31 Accordingly, in an eleventh aspect, the present
32 invention provides a method of treating a condition

1 associated with angiogenesis in a patient in need of
2 treatment thereof, said method comprising
3 administration of an antibody molecule or a nucleic
4 acid encoding said antibody molecule to said
5 patient, wherein said antibody molecule
6 specifically binds Cathepsin S but does not inhibit
7 the proteolytic activity of Cathepsin S.

8
9 According to a twelfth aspect of the invention,
10 there is provided the use of an antibody molecule or
11 a nucleic acid encoding said antibody molecule in
12 the preparation of a medicament for the treatment of
13 a condition associated with angiogenesis, wherein
14 said antibody molecule specifically binds Cathepsin
15 S but does not inhibit the proteolytic activity of
16 Cathepsin S.

17
18 These aspects of the invention may be used in the
19 treatment of any disease or condition in which
20 angiogenesis plays a part in the pathology of the
21 disease. Such conditions and diseases include, but
22 are not limited to, cancer, inflammatory conditions,
23 for example inflammatory muscle disease, rheumatoid
24 arthritis and asthma, ocular diseases, and
25 atherosclerosis.

26
27 Moreover, the demonstration that antibody molecules
28 which specifically bind Cathepsin S but do not
29 inhibit the proteolytic activity of Cathepsin S
30 nevertheless inhibit angiogenesis enables the use of
31 such antibody molecules in the treatment of other
32 conditions associated with Cathepsin S.

1
2 In a thirteenth aspect, there is provided a method
3 of treating a condition associated with activity,
4 for example aberrant activity of Cathepsin S in a
5 patient in need of treatment thereof, said method
6 comprising administration of an antibody molecule or
7 a nucleic acid encoding said antibody molecule,
8 wherein said antibody molecule specifically binds
9 Cathepsin S but does not inhibit the proteolytic
10 activity of Cathepsin S.

11
12 Also provided as a fourteenth aspect is the use of
13 an antibody molecule or a nucleic acid encoding said
14 antibody molecule in the preparation of a medicament
15 for the treatment of a condition associated with
16 aberrant activity of Cathepsin S, , wherein said
17 antibody molecule specifically binds Cathepsin S
18 but does not inhibit the proteolytic activity of
19 Cathepsin S.

20
21 As well as the treatment of tumours, the invention
22 may be used in the treatment of any condition with
23 which aberrant activity of Cathepsin S is
24 associated, in particular conditions associated with
25 aberrant expression of Cathepsin S. In one
26 embodiment, a condition is considered to be
27 associated with aberrant activity of Cathepsin S if
28 the proteolytic activity of Cathepsin S is greater
29 than in the absence of said condition, for example
30 wherein the proteolytic activity is at least 20%
31 greater, for example at least 50% greater, such as
32 at least 100% greater, at least 200% greater or at

1 least 500% greater than in the absence of said
2 condition. In one embodiment, a condition is
3 considered to be associated with aberrant expression
4 of Cathepsin S if the expression of Cathepsin S is
5 greater than in the absence of said condition, for
6 example wherein the expression is at least 20%
7 greater, for example at least 50% greater, such as
8 at least 100% greater, at least 200% greater or at
9 least 500% greater than in the absence of said
10 condition.

11

12 For example, conditions in which the invention may
13 be used include, but are not limited to
14 neurodegenerative disorders, for example Alzheimer's
15 disease and multiple sclerosis, autoimmune
16 disorders, and other diseases associated with
17 excessive, deregulated or inappropriate
18 angiogenesis.

19

20 In any of the eleventh to fourteenth aspects of the
21 present invention, any suitable anti-Cathepsin S
22 antibody molecule, which does not inhibit the
23 proteolytic activity of Cathepsin S, but which
24 inhibits angiogenesis, may be used.

25

26 Such antibody molecules and nucleic acids encoding
27 such antibody molecules constitute, a fifteenth
28 independent aspect of the invention.

29

30 In one embodiment of the fifteenth aspect of the
31 invention, the antibody molecule is a 1E4 antibody
32 or a fragment or variant thereof. Such fragments

1 and variants preferably retain the ability to
2 inhibit angiogenesis but do not have ability to
3 inhibit the proteolytic activity of Cathepsin S.

4
5 In one embodiment of the invention, the antibody
6 molecule of and for use in the invention has the
7 ability to inhibit tumour cell invasion.

8
9 According to a sixteenth aspect of the invention,
10 there is provided an antibody molecule or a nucleic
11 acid encoding said antibody molecule
12 for use in medicine, wherein said antibody molecule
13 specifically binds Cathepsin S but does not inhibit
14 the proteolytic activity of Cathepsin S

15
16 Also provided by the invention as a seventeenth
17 aspect is the use of an antibody molecule or a
18 nucleic acid encoding said antibody molecule, in the
19 preparation of a medicament for the treatment of a
20 condition associated with activity of Cathepsin S,
21 wherein said antibody molecule specifically binds
22 Cathepsin S but does not inhibit the proteolytic
23 activity of Cathepsin S in the preparation of a
24 medicament for the treatment of a condition
25 associated with activity of Cathepsin S.

26
27 Another aspect of the invention is a pharmaceutical
28 composition comprising an antibody molecule or a
29 nucleic acid encoding said antibody molecule,
30 wherein said antibody molecule specifically binds
31 Cathepsin S, but does not inhibit the proteolytic
32 activity of Cathepsin S.

1
2 Preferred features of each aspect of the invention
3 are as for each of the other aspects mutatis
4 mutandis unless the context demands otherwise.

5

6 **Detailed Description**

7

8 **Cathepsin S Inhibitors**

9

10 As described above, any suitable Cathepsin S
11 inhibitor may be used in the present invention. The
12 inhibitor may be, for example a small molecule
13 pharmaceutical, for example dipeptide α -keto- β -
14 aldehydes The dipeptide α -keto- β -aldehydes were
15 developed as potent reversible inhibitors to Cat S
16 by Walker *et al*, and have been shown to inhibit Cat
17 B and L, albeit with less efficiency (Walker *et al*,
18 2000). Another small molecule inhibitor of
19 Cathepsin S which may be used in the present
20 invention is 4-Morpholineurea-Leu-HomoPhe-
21 vinylsulphone (LHVS). Other Cathepsin S inhibitors
22 which may be used are for example the non-peptide
23 Cathepsin S inhibitors described in Thurmond *et al*,
24 J Med Chem. 2004 Sep 23;47(20):4799-801.

25

26 In another embodiment, the Cathepsin S inhibitor is
27 an antibody molecule, such as an antibody or
28 antibody fragment.

29

30

31 In the context of the present invention, an
32 "antibody" should be understood to refer to an

1 immunoglobulin or part thereof or any polypeptide
2 comprising a binding domain which is, or is
3 homologous to, an antibody binding domain.
4 Antibodies include but are not limited to
5 polyclonal, monoclonal, monospecific, polyspecific
6 antibodies and fragments thereof and chimeric
7 antibodies comprising an immunoglobulin binding
8 domain fused to another polypeptide.

9
10 Intact (whole) antibodies comprise an immunoglobulin
11 molecule consisting of heavy chains and light
12 chains, each of which carries a variable region
13 designated VH and VL, respectively. The variable
14 region consists of three complementarity determining
15 regions (CDRs, also known as hypervariable regions)
16 and four framework regions (FR) or scaffolds. The
17 CDR forms a complementary steric structure with the
18 antigen molecule and determines the specificity of
19 the antibody.

20
21 Fragments of antibodies may retain the binding
22 ability of the intact antibody and may be used in
23 place of the intact antibody. Accordingly, for the
24 purposes of the present invention, unless the
25 context demands otherwise, the term "antibodies"
26 should be understood to encompass antibody
27 fragments. Examples of antibody fragments include
28 Fab, Fab', F (ab')₂, Fd, dAb, and Fv fragments,
29 scFvs, bispecific scFvs, diabodies, linear
30 antibodies (see US patent 5, 641, 870, Example 2 ;
31 Zapata et al., Protein Eng 8 (10) : 1057-1062
32 [1995]) ; single-chain antibody molecules ; and

1 multispecific antibodies formed from antibody
2 fragments.

3

4 The Fab fragment consists of an entire L chain (VL
5 and CL), together with VH and CH1. Fab' fragments
6 differ from Fab fragments by having additional few
7 residues at the carboxy terminus of the CH1 domain
8 including one or more cysteines from the antibody
9 hinge region. The F (ab')₂ fragment comprises two
10 disulfide linked Fab fragments.

11

12 Fd fragments consist of the VH and CH1 domains.

13

14 Fv fragments consist of the VL and VH domains of a
15 single antibody.

16

17 Single-chain Fv fragments are antibody fragments
18 that comprise the VH and VL domains connected by a
19 linker which enables the scFv to form an antigen
20 binding site. (see Pluckthun in The Pharmacology of
21 Monoclonal Antibodies, vol.113, Rosenberg and Moore
22 eds., Springer-Verlag, New York, pp. 269-315 (1994)).

23

24 Diabodies are small antibody fragments prepared by
25 constructing scFv fragments (see preceding
26 paragraph) with short linkers (about 5-10 residues)
27 between the VH and VL domains such that inter-chain
28 but not intra-chain pairing of the V domains is
29 achieved, resulting in a multivalent fragment, i.e.
30 a fragment having two antigen-binding sites (see,
31 for example, EP 404 097 ; WO 93/11161 ; and
32 Hollinger et al., Proc. Natl. Acad. Sci. USA, 90 :

1 6444-6448 (1993))

2

3 Further encompassed by fragments are individual
4 CDRs.

5

6 In one embodiment of the present invention, the
7 Cathepsin S inhibitor is an antibody molecule. In
8 some embodiments of the invention, the antibody
9 molecule is an antibody molecule which inhibits the
10 proteolytic activity of Cathepsin S. An example of
11 such a suitable binding agent is the antibody 1E11
12 as described herein and in the applicant's copending
13 international patent application, WO2006/109045,
14 claiming priority from GB0507219.4 and GB0507272.3.
15 The inventors have identified the amino acid
16 sequences of the VH and VL regions of the intact
17 antibody 1E11. Furthermore, the inventors have
18 identified the six CDRs of this antibody (Seq ID
19 Nos: 1, 2, 3, 4, 5 and 6).

20

21 As described above, the antibody molecules for use
22 in any one of the first to tenth aspects of the
23 present invention are not limited to antibodies
24 having the specific sequences of the 1E11 antibody,
25 the VH, VL and the CDRs having the sequences
26 disclosed herein but also extends, for example, to
27 any other antibody which inhibits Cathepsin S
28 proteolytic activity. For example variants of 1E11,
29 which maintain the ability to inhibit the
30 proteolytic activity of Cat S may be used in some
31 embodiments of the invention. Thus, the CDR amino
32 acid sequences of the 1E11 antibody, in which one or

1 more amino acid residues are modified may also be
2 used as the CDR sequence. The modified amino acid
3 residues in the amino acid sequences of the CDR
4 variant are preferably 30% or less, more preferably
5 20% or less, most preferably 10% or less, within the
6 entire CDR. Such variants may be provided using the
7 teaching of the present application and techniques
8 known in the art. The CDRs may be carried in a
9 framework structure comprising an antibody heavy or
10 light chain sequence or part thereof. Preferably
11 such CDRs are positioned in a location corresponding
12 to the position of the CDR(s) of naturally occurring
13 VH and VL domains. The positions of such CDRs may
14 be determined as described in Kabat *et al*, Sequences
15 of Proteins of Immunological Interest, US Dept of
16 Health and Human Services, Public Health Service,
17 Nat'l Inst. of Health, NIH Publication No. 91-3242,
18 1991 and online at www.kabatdatabase.com
19 <http://immuno.bme.nwu.edu>.

20
21 Furthermore, modifications may alternatively or
22 additionally be made to the Framework Regions of the
23 variable regions. Such changes in the framework
24 regions may improve stability and reduce
25 immunogenicity of the antibody.

26
27 However, as described above and as shown in the
28 Examples, the present inventors have also shown that
29 anti-angiogenic effects may be achieved using
30 antibody molecules which bind Cathepsin S but which
31 do not inhibit the proteolytic activity of Cathepsin

1 S. Such antibody molecules may be used in any aspect
2 of the present invention.

3

4 Antibodies for use in the invention herein include
5 "chimeric" antibodies in which a portion of the
6 heavy and/or light chain is identical with or
7 homologous to corresponding sequences in antibodies
8 derived from a particular species or belonging to a
9 particular antibody class or subclass, while the
10 remainder of the chain(s) is identical with or
11 homologous to corresponding sequences in antibodies
12 derived from another species or belonging to another
13 antibody class or subclass, as well as fragments of
14 such antibodies, so long as they exhibit the desired
15 biological activity (see U. S. Patent No. 4, 816,
16 567 ; and Morrison *et al.*, Proc. Natl. Acad. Sci.
17 USA, 81 : 6851-6855 (1984)). Chimeric antibodies of
18 interest herein include "primatized" antibodies
19 comprising variable domain antigen-binding sequences
20 derived from a non-human primate(e. g. Old World
21 Monkey, Ape etc), and human constant region
22 sequences.

23

24 Antibody molecules for use in the present invention
25 may be produced in any suitable way, either
26 naturally or synthetically. Such methods may
27 include, for example, traditional hybridoma
28 techniques (Kohler and Milstein (1975) Nature, 256
29 :495-499), recombinant DNA techniques (see e.g. U.
30 S. Patent No. 4,816, 567), or phage display
31 techniques using antibody libraries (see e.g.
32 Clackson *et al.* (1991) Nature, 352: 624-628 and

1 Marks *et al.* (1992) *Bio/ Technology*, 10: 779-783).
2 Other antibody production techniques are described
3 in *Using Antibodies: A Laboratory Manual*, eds.
4 Harlow *et al.*, Cold Spring Harbor Laboratory, 1999.
5
6 Traditional hybridoma techniques typically involve
7 the immunisation of a mouse or other animal with an
8 antigen in order to elicit production of lymphocytes
9 capable of binding the antigen. The lymphocytes are
10 isolated and fused with a myeloma cell line to form
11 hybridoma cells which are then cultured in
12 conditions which inhibit the growth of the parental
13 myeloma cells but allow growth of the antibody
14 producing cells. The hybridoma may be subject to
15 genetic mutation, which may or may not alter the
16 binding specificity of antibodies produced.
17 Synthetic antibodies can be made using techniques
18 known in the art (see, for example, Knappik *et al.*,
19 *J. Mol. Biol.* (2000) 296, 57-86 and Krebs *et al.*, *J.*
20 *Immunol. Meth.* (2001) 2154 67-84.
21
22 Modifications may be made in the VH, VL or CDRs of
23 the binding members, or indeed in the FRS using any
24 suitable technique known in the art. For example,
25 variable VH and/or VL domains may be produced by
26 introducing a CDR, e.g. CDR3 into a VH or VL domain
27 lacking such a CDR. Marks *et al.* (1992) *Bio/*
28 *Technology*, 10: 779-783 describe a shuffling
29 technique in which a repertoire of VH variable
30 domains lacking CDR3 is generated and is then
31 combined with a CDR3 of a particular antibody to
32 produce novel VH regions. Using analogous

1 techniques, novel VH and VL domains comprising CDR
2 derived sequences of the present invention may be
3 produced.

4
5 Accordingly, antibody molecules for use in the
6 invention may be produced by a method comprising:
7 (a) providing a starting repertoire of nucleic acids
8 encoding a variable domain, wherein the variable
9 domain includes a CDR1, CDR2 or CDR3 to be replaced
10 or the nucleic acid lacks an encoding region for
11 such a CDR; (b) combining the repertoire with a
12 donor nucleic acid encoding an amino acid sequence
13 having the sequence as shown as Seq ID No: 1, 2, 3,
14 4, 5 or 6 herein such that the donor nucleic acid
15 is inserted into the CDR region in the repertoire so
16 as to provide a product repertoire of nucleic acids
17 encoding a variable domain; (c) expressing the
18 nucleic acids of the product repertoire; (d)
19 selecting a specific antigen-binding fragment
20 specific for CatS; and (e) recovering the specific
21 antigen-binding fragment or nucleic acid encoding
22 it. The method may include an optional step of
23 testing the antibody molecule for ability to inhibit
24 the proteolytic activity of Cathepsin S.

25
26 Alternative techniques of producing antibodies for
27 use in the invention may involve random mutagenesis
28 of gene(s) encoding the VH or VL domain using, for
29 example, error prone PCR (see Gram *et al*, 1992,
30 P.N.A.S. 89 3576-3580. Additionally or
31 alternatively, CDRs may be targeted for mutagenesis
32 e.g. using the molecular evolution approaches

1 described by Barbas et al 1991 PNAS 3809-3813 and
2 Scier 1996 J Mol Biol 263 551-567.

3

4 Having produced such variants, antibodies and
5 fragments may be tested for binding to Cat S and for
6 the ability to inhibit the proteolytic activity of
7 Cathepsin S.

8

9 As described herein, the inventors have demonstrated
10 that antibody molecules which may be used in some
11 aspects of the present invention have an anti-
12 proteolytic effect. Furthermore anti-invasive and
13 anti-angiogenic activity has also been demonstrated,
14 as described in the Examples. This therefore
15 enables the use of the antibody molecules of the
16 invention as active therapeutic agents. An antibody
17 molecule for use in the invention may be a "naked"
18 antibody molecule. A "naked" antibody molecule is
19 an antibody molecule which is not conjugated with an
20 "active therapeutic agent". In the context of the
21 present application, an "active therapeutic agent"
22 is a molecule or atom which is conjugated to an
23 antibody moiety (including antibody fragments, CDRs
24 etc) to produce a conjugate. Examples of such
25 "active therapeutic agents" include drugs, toxins,
26 radioisotopes, immunomodulators, chelators, boron
27 compounds , dyes etc.

28

29 **Immunoconjugates**

30

31 In another embodiment of the invention, an antibody
32 molecule for use in some embodiments of the

1 invention may be in the form of an immunoconjugate,
2 comprising an antibody fragment conjugated to an
3 "active therapeutic agent". The therapeutic agent
4 may be a chemotherapeutic agent or another molecule.

5
6 Methods of producing immunoconjugates are well known
7 in the art; for example, see U. S. patent No.
8 5,057,313, Shih *et al.*, *Int. J. Cancer* 41: 832-839
9 (1988); Shih *et al.*, *Int. J. Cancer* 46: 1101-1106
10 (1990), Wong, *Chemistry Of Protein Conjugation And*
11 *Cross-Linking* (CRC Press 1991); Upeslakis *et al.*,
12 "Modification of Antibodies by Chemical Methods" in
13 *Monoclonal Antibodies: Principles And Applications*,
14 Birch *et al.* (eds.), pages 187-230 (Wiley-Liss, Inc.
15 1995); Price, "Production and Characterization of
16 Synthetic Peptide-Derived Antibodies," in *Monoclonal*
17 *Antibodies: Production, Engineering And Clinical*
18 *Application*, Ritter *et al.* (eds.), pages 60-84
19 (Cambridge University Press 1995).

20
21 In those aspects of the present invention, where it
22 is desired to employ a combination of a Cathepsin S
23 inhibitor and a chemotherapeutic, an immunoconjugate
24 comprising an anti-Cathepsin S antibody and a
25 chemotherapeutic agent may be used.

26
27 Such immunoconjugates will have a number of
28 advantages over conventional treatments. The
29 demonstration that Cathepsin S is localised on
30 tumour cells enables the use of such
31 immunoconjugates to target chemotherapeutic agents
32 to tumour cells. The targeted chemotherapeutic agent

1 may have its cytotoxic effect. Secondly, as
2 described above, it has been found that
3 conventionally used chemotherapeutics cause
4 upregulation of Cathepsin S on the cell surface of
5 tumour cells, for example colorectal tumour cells.
6 By employing an immunoconjugate of an anti-Cathepsin
7 S antibody and a chemotherapeutic agent, the
8 Cathepsin S antibody part of the immunoconjugate is
9 expected to counteract this effect. Moreover, the
10 antibody portion of the immunoconjugate would be
11 expected to have a longer active half life in the
12 body than the chemotherapeutic agent and so may
13 counteract the effects of the Cathepsin S
14 upregulation caused by the chemotherapeutic agent to
15 which it was attached.

16

17 An immunoconjugate of an anti-Cathepsin S antibody
18 molecule and a chemotherapeutic agent forms a
19 further aspect of the present invention. In one
20 embodiment, the antibody molecule is cleavable from
21 the chemotherapeutic agent by a protease, for
22 example, a Cathepsin.

23

24 The antibody molecules for use in the invention may
25 comprise further modifications. For example the
26 antibodies can be glycosylated, pegylated, or linked
27 to albumin or a nonproteinaceous polymer. The
28 antibody molecule may be in the form of an
29 immunoconjugate.

30

31 The ability of an agent, for example a small
32 molecule or antibody, to inhibit the proteolytic

1 activity of Cathepsin S may be tested using any
2 suitable method. For example a fluorimetric assay
3 may be used. In such an assay, any suitable
4 fluorogenic substrate may be used, for example Cbz-
5 Phe-Arg-AMC. An agent is considered to inhibit the
6 proteolytic activity of Cathepsin S if it has the
7 ability to inhibit its activity by a statistically
8 significant amount. For example, in one embodiment,
9 an agent for use as the Cathepsin S inhibitor is
10 able to inhibit the inhibitory activity by at least
11 10%, for example at least 25%, 50%, 70%, 80% or 90%,
12 when compared to an appropriate control antibody.

13

14 The ability of an agent to inhibit tumour cell
15 invasion may be tested using any suitable invasion
16 assay known in the art. For example, such ability
17 may be tested using a modified Boyden chamber as is
18 known in the art. The antibody molecule may be
19 tested using any suitable tumour cell line, for
20 example a prostate carcinoma cell line, e.g. pC3, an
21 astrocytoma cell line e.g. U251mg, a colorectal
22 carcinoma cell line, e.g. HCT116, or a breast cancer
23 cell line, e.g. MDA-MB-231 or MCF7. An agent may be
24 considered to inhibit tumour cell invasion if it has
25 the ability to inhibit invasion by a statistically
26 significant amount. For example, in one embodiment,
27 an agent for use as the Cathepsin S inhibitor is
28 able to inhibit invasion by at least 10%, for
29 example at least 25%, 50%, 70%, 80% or 90% when
30 compared to an appropriate control antibody.

31

1 The ability of an agent to inhibit angiogenesis may
2 be tested using any suitable assay known in the art.
3 For example, such ability may be tested using the
4 assays described in the Assay section and/or as
5 described in the Examples, for example a Matrigel
6 based assay.

7
8 In certain aspects of the invention, for example in
9 assays to determine the presence of tumour cells in
10 a population of cells or in diagnostic techniques to
11 determine the presence of cells in vivo, labelled
12 antibody molecules may be used. Labels which may be
13 used include radiolabels, enzyme labels such as
14 horseradish peroxidase, alkaline phosphatase, or
15 biotin.

16

17 **Nucleic Acid**

18

19 Nucleic acid for use in the present invention may
20 comprise DNA or RNA. It may be produced
21 recombinantly, synthetically, or by any means
22 available to those in the art, including cloning
23 using standard techniques.

24

25 The nucleic acid may be inserted into any
26 appropriate vector, for example a virus (e. g.
27 vaccinia virus, adenovirus, etc.), baculovirus; yeast
28 vector, phage, chromosome, artificial chromosome,
29 plasmid, or cosmid DNA. Vectors may be used to
30 introduce the nucleic acids into a host cell, which
31 may be prokaryotic or eukaryotic.

32

1 For further details relating to known techniques and
2 protocols for manipulation of nucleic acid, for
3 example, in preparation of nucleic acid constructs,
4 mutagenesis, sequencing, introduction of DNA into
5 cells and gene expression, and analysis of proteins,
6 see, for example, Current Protocols in Molecular
7 Biology, 5th ed., Ausubel et al. eds., John Wiley &
8 Sons, 2005 and, Molecular Cloning: a Laboratory
9 Manual: 3rd edition Sambrook et al., Cold Spring
10 Harbor Laboratory Press, 2001.

11

12 **Chemotherapeutic Agents**

13

14 As described above, in some embodiments of the
15 invention, chemotherapeutic agents may be used, for
16 example in combination treatment regimes with
17 Cathepsin S inhibitors. In such embodiments, any
18 suitable chemotherapeutic agent may be used in the
19 present invention. For example agents which may be
20 used include antimetabolites, nucleoside analogs,
21 thymidylate synthase inhibitors, platinum cytotoxic
22 agents or topoisomerase inhibitors. Examples of
23 thymidylate synthase inhibitors which may be used in
24 the invention include 5-FU, MTA and TDX. In one
25 embodiment, the thymidylate synthase inhibitor is 5-
26 FU. An example of an antimetabolite which may be
27 used is tomudex (TDX). Examples of platinum
28 cytotoxic agents which may be used include cisplatin
29 and oxaliplatin. In one embodiment of the invention,
30 the chemotherapeutic agent is cisplatin. Any
31 suitable topoisomerase inhibitor may be used in the
32 present invention. Examples of nucleoside analogs

1 which may be used include but are not limited to
2 gemcitabine and cytarabine.

3

4 In a particular embodiment of the invention, the
5 chemotherapeutic agent is a topoisomerase inhibitor.
6 Any suitable topoisomerase inhibitor may be used in
7 the present invention. In a particular embodiment,
8 the topoisomerase inhibitor is a topoisomerase I
9 inhibitor, for example a camptothecin. A suitable
10 topoisomerase I inhibitor, which may be used in the
11 present invention is irinotecan (CPT-11) or its
12 active metabolite SN-38. CPT-11 specifically acts in
13 the S phase of the cell cycle by stabilizing a
14 reversible covalent reaction intermediate, referred
15 to as a cleavage or cleavage complex and may also
16 induce G₂-M cell cycle arrest.

17

18 Chemotherapeutic agents which may be used in the
19 present invention in addition or instead of the
20 specific agents recited above, may include
21 alkylating agents; alkyl sulfonates; aziridines;
22 ethylenimines; methylamelamines; nitrogen mustards;
23 nitrosureas; anti-metabolites; folic acid analogues;
24 purine analogs; pyrimidine analogs; androgens; anti-
25 adrenals; folic acid replenishers; aceglatone;
26 aldophosphamide glycoside; aminolevulinic acid;
27 amsacrine; bestabucil; bisantrene; edatraxate;
28 defofamine; demecolcine; diaziquone; elfomithine;
29 elliptinium acetate; etoglucid; gallium nitrate;
30 hydroxyurea; lentinan; ionidamine; mitoguazone;
31 mitoxantrone

32

1 In a particular embodiment of the invention, the
2 chemotherapeutic agent is a fluoropyrimidine, e.g.
3 5-FU, or a metabolite thereof. 5-FU is used in the
4 treatment of many cancers, including
5 gastrointestinal, breast and head and neck cancers.
6 5-FU is converted intracellularly to
7 fluorodeoxyuridine monophosphate FdUMP, which,
8 together with 5,10-methylene tetrahydrofolate
9 (CH_2THF) forms a stable ternary complex with
10 thymidylate synthase (TS), resulting in enzyme
11 inhibition. TS catalyses the reductive methylation
12 of deoxyuridine monophosphate (dUMP) by CH_2THF to
13 produce deoxythymidine monophosphate (dTMP) and
14 dihydrofolate (Longley et al Nat Rev Cancer, 3:330-
15 338, 2003). As this reaction provides the sole *de*
16 *novo* intracellular source of dTMP, which is
17 essential for DNA replication and repair, TS
18 inhibition results in DNA damage. Non-TS-directed
19 mechanisms of cytotoxicity have also been described
20 for 5-FU, such as misincorporation of
21 fluoronucleotides into DNA and RNA (Longley et al
22 Nat Rev Cancer, 3:330-338, 2003).
23
24 Where reference is made to specific chemotherapeutic
25 agents, it should be understood that analogues
26 including biologically active derivatives and
27 substantial equivalents thereof, which retain the
28 antitumour activity of the specific agents, may be
29 used.
30

1 **Treatment**

2 Treatment" includes any regime that can benefit a
3 human or non-human animal. The treatment may be in
4 respect of an existing condition or may be
5 prophylactic (preventative treatment). Treatment may
6 include curative, alleviation or prophylactic
7 effects.

8
9 "Treatment of cancer" includes treatment of
10 conditions caused by cancerous growth and/or
11 vascularisation and includes the treatment of
12 neoplastic growths or tumours. Examples of tumours
13 that can be treated using the invention are, for
14 instance, sarcomas, including osteogenic and soft
15 tissue sarcomas, carcinomas, e.g., breast-, lung-,
16 bladder-, thyroid-, prostate-, colon-, rectum-,
17 pancreas-, stomach-, liver-, uterine-, prostate ,
18 cervical and ovarian carcinoma, non-small cell lung
19 cancer, hepatocellular carcinoma, lymphomas,
20 including Hodgkin and non-Hodgkin lymphomas,
21 neuroblastoma, melanoma, myeloma, Wilms tumor, and
22 leukemias, including acute lymphoblastic leukaemia
23 and acute myeloblastic leukaemia, astrocytomas,
24 gliomas and retinoblastomas.

25
26 In one embodiment, the present invention may be used
27 to treat colorectal tumours.

28
29 The invention may be particularly useful in the
30 treatment of existing cancer and in the prevention
31 of the recurrence of cancer after initial treatment
32 or surgery.

1

2 **Pharmaceutical Compositions**

3

4 Pharmaceutical compositions according to the present
5 invention, and for use in accordance with the
6 present invention may comprise, in addition to
7 active ingredients, a pharmaceutically acceptable
8 excipient, a carrier, buffer stabiliser or other
9 materials well known to those skilled in the art
10 (see, for example, (Remington: the Science and
11 Practice of Pharmacy, 21st edition, Gennaro AR, *et*
12 *al*, eds., Lippincott Williams & Wilkins, 2005.).
13 Such materials may include buffers such as acetate,
14 Tris, phosphate, citrate, and other organic acids ;
15 antioxidants; preservatives; proteins, such as serum
16 albumin, gelatin, or immunoglobulins ; hydrophilic
17 polymers such as polyvinylpyrrolidone ; amino acids
18 such as glycine, glutamine, asparagine, histidine,
19 arginine, or lysine ; carbohydrates; chelating
20 agents; tonicifiers; and surfactants.

21

22 The pharmaceutical compositions may also contain one
23 or more further active compounds selected as
24 necessary for the particular indication being
25 treated, preferably with complementary activities
26 that do not adversely affect the activity of the
27 composition of the invention. For example, in the
28 treatment of cancer, where a combination regime is
29 being used, in addition to a Cathepsin S inhibitor,
30 for example an anti Cathepsin S antibody molecule
31 and a chemotherapeutic agent, the formulation or kit
32 may comprise an additional component, for example a

1 second or further Cathepsin S inhibitor, a second or
2 further chemotherapeutic agent, or an antibody to a
3 target other than Cathepsin S, for example to a
4 growth factor which affects the growth of a
5 particular cancer.

6
7 The active ingredients (e.g. antibody molecules
8 and/or chemotherapeutic agents) may be administered
9 via microspheres, microcapsules liposomes, other
10 microparticulate delivery systems. For example,
11 active ingredients may be entrapped within
12 microcapsules which may be prepared, for example, by
13 coacervation techniques or by interfacial
14 polymerization, for example, hydroxymethylcellulose
15 or gelatin microcapsules and poly-
16 (methylemethacrylate) microcapsules, respectively, in
17 colloidal drug delivery systems (for example,
18 liposomes, albumin microspheres, microemulsions,
19 nano-particles and nanocapsules) or in
20 macroemulsions. For further details, see Remington:
21 the Science and Practice of Pharmacy, 21st edition,
22 Gennaro AR, *et al*, eds., Lippincott Williams &
23 Wilkins, 2005.

24
25 Sustained-release preparations may be used for
26 delivery of active agents. Suitable examples of
27 sustained-release preparations include semi-
28 permeable matrices of solid hydrophobic polymers
29 containing the antibody, which matrices are in the
30 form of shaped articles, e. g. films, suppositories
31 or microcapsules. Examples of sustained-release
32 matrices include polyesters, hydrogels (for example,

1 poly (2-hydroxyethyl-methacrylate), or poly
2 (vinylalcohol)), polylactides (U. S. Pat. No. 3,
3 773, 919), copolymers of L-glutamic acid and ethyl-L
4 glutamate, non-degradable ethylene-vinyl acetate,
5 degradable lactic acid-glycolic acid copolymers, and
6 poly-D- (-)-3-hydroxybutyric acid.

7
8 As described above nucleic acids may also be used in
9 methods of treatment. Nucleic acid for use in the
10 invention may be delivered to cells of interest
11 using any suitable technique known in the art.
12 Nucleic acid (optionally contained in a vector) may
13 be delivered to a patient's cells using in vivo or
14 ex vivo techniques. For in vivo techniques,
15 transfection with viral vectors (such as adenovirus,
16 Herpes simplex I virus, or adeno-associated virus)
17 and lipid-based systems (useful lipids for lipid-
18 mediated transfer of the gene are DOTMA, DOPE and
19 DC-Chol, for example) may be used (see for example,
20 Anderson et al., Science 256 : 808-813 (1992). See
21 also WO 93/25673).

22
23 In ex vivo techniques, the nucleic acid is
24 introduced into isolated cells of the patient with
25 the modified cells being administered to the patient
26 either directly or, for example, encapsulated within
27 porous membranes which are implanted into the
28 patient (see, e. g. U. S. Patent Nos. 4, 892, 538
29 and 5, 283, 187). Techniques available for
30 introducing nucleic acids into viable cells may
31 include the use of retroviral vectors, liposomes,
32 electroporation, microinjection, cell fusion, DEAE-

1 dextran, the calcium phosphate precipitation method,
2 etc.

3
4 The binding member, agent, product or composition
5 may be administered in a localised manner to a
6 tumour site or other desired site or may be
7 delivered in a manner in which it targets tumour or
8 other cells. Targeting therapies may be used to
9 deliver the active agents more specifically to
10 certain types of cell, by the use of targeting
11 systems such as antibody or cell specific ligands.
12 Targeting may be desirable for a variety of reasons,
13 for example if the agent is unacceptably toxic, or
14 if it would otherwise require too high a dosage, or
15 if it would not otherwise be able to enter the
16 target cells.

17
18 **Kits**

19
20 The invention further extends to a pharmaceutical
21 kit comprising a Cathepsin S inhibitor and a
22 chemotherapeutic agent for combination therapy by
23 simultaneous, sequential or separate administration
24 of the Cathepsin S inhibitor and chemotherapeutic
25 agent, optionally with instructions for the
26 administration of (a) and (b) separately,
27 sequentially or simultaneously.

28
29 **Dose**

30
31 The Cathepsin S inhibitors, anti-Cathepsin S
32 antibodies and/or chemotherapeutic agents of and for

1 use in the invention, as appropriate, are suitably
2 administered to an individual in a "therapeutically
3 effective amount", this being sufficient to show
4 benefit to the individual. The actual dosage
5 regimen will depend on a number of factors including
6 the condition being treated, its severity, the
7 patient being treated, the agents being used, and
8 will be at the discretion of the physician.

9
10 In embodiments, in which both a Cathepsin S
11 inhibitor and a chemotherapeutic agent are employed
12 in a combination treatment regime, the Cathepsin S
13 inhibitor and chemotherapeutic agent may be
14 administered simultaneously, separately or
15 sequentially with the chemotherapeutic agent. Where
16 administered separately or sequentially, they may be
17 administered within any suitable time period e. g.
18 within 1, 2, 3, 6, 12, 24, 48 or 72 hours of each
19 other. In preferred embodiments, they are
20 administered within 6, preferably within 2, more
21 preferably within 1, most preferably within 20
22 minutes of each other.

23
24 In one embodiment, in which both a Cathepsin S
25 inhibitor and a chemotherapeutic agent are employed
26 in a combination treatment regime, the Cathepsin S
27 inhibitor and chemotherapeutic agent are
28 administered in doses which produce a synergistic
29 effect.

30
31 i.e. administered in a potentiating ratio. The term
32 "potentiating ratio" in the context of the present

1 invention is used to indicate that the Cathepsin S
2 inhibitor and chemotherapeutic agent are present in
3 a ratio such that the cytotoxic activity of the
4 combination is greater than that of either component
5 alone or of the additive activity that would be
6 predicted for the combinations based on the
7 activities of the individual components.

8
9 Thus in a potentiating ratio, the individual
10 components act synergistically.

11
12 Synergism may be defined using a number of methods.

13
14 For example, synergism may be defined as an RI of
15 greater than unity using the method of Kern (Cancer
16 Res, 48: 117-121, 1988) as modified by Romanelli
17 (Cancer Chemother Pharmacol, 41: 385-390, 1998). The
18 RI may be calculated as the ratio of expected cell
19 survival (Se/defined as the product of the survival
20 observed with drug A alone and the survival observed
21 with drug B alone) to the observed cell survival
22 (Sobs) for the combination of A and B
23 ($RI = S_{exp} / S_{obs}$).

24
25 Synergism may then be defined as an RI of greater
26 than unity.

27
28 In another method, synergism may be determined by
29 calculating the combination index (CI) according to
30 the method of Chou and Talalay (Adv Enzyme Regul,
31 22: 27-55, 1984). CI values of 1, < 1, and > 1
32 indicate additive, synergistic and antagonistic

1 effects respectively.

2

3 In an embodiment of the invention, the Cathepsin S
4 inhibitor and the chemotherapeutic agent are present
5 in concentrations sufficient to produce a CI of less
6 than 1, preferably less than 0.85.

7

8 Synergism is preferably defined as an RI of greater
9 than unity using the method of Kern as modified by
10 Romaneli (1998a, b). The RI may be calculated as the
11 ratio of expected cell survival (Sep, defined as the
12 product of the survival observed with drug A alone
13 and the survival observed with drug B alone) to the
14 observed cell survival (Sobs) for the combination of
15 A and B ($RI = Se/Sobs$). Synergism may then be defined
16 as an RI of greater than unity.

17

18 In an embodiment of the invention, said Cathepsin S
19 inhibitor and chemotherapeutic agent are provided in
20 concentrations sufficient to produce an RI of
21 greater than 1.5, for example greater than 2.0, such
22 as greater than 2.25.

23

24 In one embodiment of the invention, the combined
25 medicament thus produces a synergistic effect when
26 used to treat tumour cells.

27

28 **Assays**

29

30 The determination that Cathepsin S is upregulated on
31 the cell surface of tumour cells in response to
32 chemotherapeutic agents enables the provision of

1 assays to determine whether or not a
2 chemotherapeutic agent is having such an effect in a
3 tissue of a patient.
4 Thus, in a further aspect of the present invention,
5 there is provided a method for evaluating in vitro
6 the response of tumour cells from a subject to the
7 presence of a chemotherapeutic agent to predict
8 response of the tumour cells in vivo to treatment
9 with the chemotherapeutic agent which method
10 comprises : (a) providing an in vitro sample from a
11 subject containing tumour cells; (b) exposing a
12 portion of said sample of tumour cells to said
13 chemotherapeutic agent; (c) comparing expression of
14 Cathepsin S on the surface of said cells with
15 expression of Cathepsin S in a control portion of
16 said sample which has not been exposed to said
17 chemotherapeutic agent; wherein enhanced expression
18 in the portion of sample exposed to said
19 chemotherapeutic agent is indicative of sensitivity
20 to said chemotherapeutic agent.

21
22 In preferred embodiments of this aspect of the
23 invention, expression in the sample exposed to said
24 chemotherapeutic agent is considered to be enhanced
25 if the expression is at least 3-fold, preferably at
26 least 4-fold, more preferably at least 5-fold, even
27 more preferably at least 7-fold, yet more preferably
28 at least 10-fold, most preferably at least 12-fold
29 that of the one or more genes in the control portion
30 of said sample which has not been exposed to said
31 chemotherapeutic agent.

32

1 Such an assay may be used to determine the
2 suitability of combination therapy using
3 chemotherapeutic agent and a Cathepsin S inhibitor
4 in a particular patient.

5
6 The method may alternatively or additionally be used
7 to monitor disease progression, for example using
8 biopsy samples at different times. In such
9 embodiments, instead of comparing the expression of
10 Cathepsin S against a control sample which has not
11 been exposed to said chemotherapeutic agent, the
12 expression of Cathepsin S is compared against a
13 sample obtained from the same tissue at an earlier
14 time point, for example from days, weeks or months
15 earlier.

16
17 The nature of the tumour or cancer will determine
18 the nature of the sample which is to be used in the
19 methods of the invention. The sample may be, for
20 example, a sample from a tumour tissue biopsy, bone
21 marrow biopsy or circulating tumour cells in e. g.
22 blood. Alternatively, e. g. where the tumour is a
23 gastrointestinal tumour, tumour cells may be
24 isolated from faeces samples. Other sources of
25 tumour cells may include plasma, serum,
26 cerebrospinal fluid, urine, interstitial fluid,
27 ascites fluid etc.

28
29 For example, solid tumours may be collected in
30 complete tissue culture medium with antibiotics.

31
32 Cells may be manually teased from the tumour

1 specimen or, where necessary, are enzymatically
2 disaggregated by incubation with collagenase/DNAse
3 and suspended in appropriate media containing, for
4 example, human or animal sera.

5
6 In other embodiments, biopsy samples may be isolated
7 and frozen or fixed in fixatives such as formalin.
8 The samples may then be tested for expression levels
9 of genes at a later stage.

10

11

12 In some embodiments of the invention, the effect of
13 a Cathepsin S antibody molecule or combination
14 treatments on cell viability, proliferation or
15 angiogenesis may be desired to be testes. These
16 effects may be tested using any methods known in the
17 art. Some suitable assays are described in the
18 Examples. Others include:

19

20 **Cell viability and proliferation assays**

21

22 Cytotoxic and proliferative effects of an antibody
23 on tumour cells may be tested, for example, on
24 U251mg astrocytoma cells can be tested as previously
25 described. Briefly, cells are added to a final
26 concentration of 1×10^4 cells/200 μ l per well of a
27 96-well microtiter plate (Corning Costar).

28 Appropriate concentrations of monoclonal antibody
29 (100 nM) or vehicle-only control media are added.
30 Plates are incubated at 37°C and 5% CO₂ for 24, 48,
31 72 and 96 hrs respectively. After incubation, 10 μ l
32 of 10 mg/ml MTT is added and incubated for a further

1 2 h at 37°C and 5% CO₂. The medium is carefully
2 removed and formazan crystals dissolved in 100
3 µl/well of DMSO. Absorbance is measured as described
4 above and the results expressed as the percentage of
5 cell viability and proliferation relative to each
6 vehicle-only control. All tests are performed in
7 quadruplicate.

8
9 A number of assays may be used to investigate the
10 effects of a molecule on angiogenesis. In the
11 Examples, the aortic ring model is described.
12 Others include:

14 **Wound Assay**

15 This *in vitro* migration is a modified version of the
16 method described by Ashton et al (1999). HMEC-1 is
17 plated into individual chambers on a glass slide and
18 grown to 90% confluence overnight. The medium is
19 removed and the monolayer wounded. The monolayer is
20 re-supplemented with fresh medium and the required
21 volume of antibodies added to give the required
22 final concentration.

23
24 Slides are removed at fixed time points until
25 complete closure of the wound, then fixed in 4% PBS
26 buffered paraformaldehyde. The extent of "wound"
27 closure is blindly assessed microscopically by an
28 independent investigator and quantified using a
29 calibrated eyepiece graticule (1mm/100µm graduation)
30 at 20x magnification (Olympus BX 50). The extent of
31 closure in the antibody treated slides is compared
32 to time matched sham treated controls and the %

1 inhibition of wound closure compared to time matched
2 controls calculated.

3

4 **Sponge Assay**

5

6 Polyether sponges are subcutaneously implanted in
7 C57 black mice and injected on alternate days with
8 10 ng bFGF or 10 ng bFGF+ antibodies. After 14 days
9 of treatment, sponges are harvested, sectioned and
10 stained with H&E (A).

11

12 **Oxygen-induced retinopathy (OIR) neonatal mouse** 13 **model**

14

15 The animal model of oxygen-induced retinopathy
16 employed in this investigation is that described by
17 Smith et al and now widely used for studies testing
18 anti-angiogenic therapies for retinal
19 neovascularisation (Smith et al., 1994). Litters of
20 postnatal mice and their nursing dams are placed in
21 75% oxygen at postnatal day 7 (P7) and returned to
22 room air at P12. During the 5-day period of
23 hyperoxia the central retinal capillary beds undergo
24 vascular regression and halted development. Upon
25 return to room air the central retina becomes
26 measurably hypoxic with an associated increase in
27 vasogenic growth factors of the VEGF family. An
28 aggressive neovascular response ensues with extra-
29 retinal new vessels developing by P15.
30 Neovascularization is maximal at P17 and regresses
31 after P21.

32

1 Unfixed neural retina are dissected and subjected to
2 ultrasonic disruption in RIPA buffer. For
3 quantification of HP-protein binding, retinal
4 samples from P13 and P17 mice are prepared for a HP
5 competitive enzyme-linked immunosorbent assay
6 (ELISA) methodology. Briefly, 100 μ l of sample or
7 standard in duplicate are serially diluted in PBS/5%
8 Tween 20 in a 96-well polystyrene assay plate
9 (Corning Inc., Corning, NY) and incubated with 25 μ l
10 of rabbit polyclonal antibody against HP (1:3300 in
11 PBS/5% Tween) for 1 hour at 37°C. The contents of
12 each well is then transferred to NUNC C96 Maxisorp
13 plates that are precoated with solid phase antigen
14 (1:5000 in carbonate buffer, pH 9.6) overnight at
15 4°C and blocked with 1% gelatin for 1 hour. The
16 competition between solid phase and soluble antigens
17 proceeds for 1 hour at 37°C, after which the wells
18 are washed 4 x 5minutes with PBS/5% Tween, and 100
19 μ l of 1:2000 alkaline-phosphatase goat anti-rabbit
20 IgG (Sigma-Aldrich) is added to each well. Plates
21 are then washed 4 x 5minutes with PBS/5% Tween, and
22 100 μ l of 1 mg/ml of the alkaline-phosphatase
23 substrate, 4-nitrophenyl phosphate disodium salt
24 hexahydrate (Sigma-Aldrich) dissolved in 10%
25 diethanolamine buffer (pH 9.6) is added to each
26 well. The color development of the subsequent
27 reaction is measured at 405 nm every 5 minutes for 2
28 hours on a Safire microplate reader (Tecan
29 Instruments) and reaction kinetics analyzed using
30 Magellan 3 software. Sample HP-1 binding is
31 determined by comparison to a Lineweaver-Burk enzyme
32 kinetic standard curve, and is expressed as a

1 proportion of sample protein concentration. The
2 solid-phase antigen used in the assay is HP, which
3 is reductively bound to bovine serum albumin.
4 Statistical analysis is conducted using the
5 Student's t-test and a P value <0.05 is considered
6 significant.

7
8 The invention will now be described further in the
9 following non-limiting examples. Reference is made
10 to the accompanying drawings in which:

11
12 Figure 1 illustrates analysis of CatS RNA expression
13 in HCT116 cells and RKO cells, with / without a 48
14 hour CPT11 treatment.

15
16 Figure 2 illustrates Western blot analysis of CatS
17 and gamma tubulin protein expression in HCT116 +/-
18 cells, RKO +/- cells and HT29 p53 mutant cells with
19 / without a 48 hour CPT11 treatment.

20
21 Figure 3 illustrates flow cytometric analysis of
22 CatS cell surface expression on RKO+/+ cells
23 untreated (3A) and CPT11 48 hour treated (3B) and
24 HCT116+/+ cells untreated (3C) and CPT11 48 hour
25 treated (3D). Cells were stained with CatS
26 monoclonal antibody (Green - lower peaks on each
27 graph (diagonal stripes)) and Isotype matched
28 control (Red- high peak on left on each graph
29 (vertical stripes)).

30
31 Figure 4 illustrates flow cytometric analysis of
32 CatS cell surface expression on H630 cells untreated

1 (4A) and CPT11 48 hour treated (4B). Cells were
2 stained with CatS monoclonal antibody (Green -
3 double peak on each graph (diagonal stripes)) and
4 isotype matched control (Red - single higher peak on
5 left of each graph(vertical stripes)).

6
7 Figure 5 illustrates a photograph showing the
8 results of immunocytochemical analysis using the
9 1E11 CatS monoclonal antibody, revealing that CatS
10 expression is localised to the cell surface on
11 HCT116 cells. The right panel shows the results of
12 flow cytometry analysis, where a 38% increase in
13 fluorescent intensity was observed in HCT116 cells
14 stained for CatS in comparison to the isotype
15 control

16
17 Figure 6 illustrates the results of
18 immunohistochemical detection of CatS expression in
19 colorectal carcinoma biopsies And in normal spleen
20 and colon samples.

21
22 Figure 7 illustrates analysis of CatS and $\beta 3$
23 integrin RNA expression in HCT116 +/+, colorectal
24 cancer cells compared to control (non-tumour cells).
25 RNA levels were analysed following 35 cycle of PCR.

26
27 Fig 8a illustrates immunohistochemical detection of
28 CatS 1E11 mAb localisation to tumour in untreated
29 and treated (CatS 1E11 + 5Fu (IC50)) xenograft
30 models. Dark (Brown) staining indicates positive
31 staining.

32

1 Fig 8b illustrates immunohistochemical detection of
2 CatS 1E11 mAb localisation to tumour in untreated
3 and treated (CatS 1E11 + 5Fu (IC50)) xenograft
4 models. Dark (Brown) staining indicates positive
5 localisation.

6

7 Fig 9 shows photographs of gut and liver sections
8 after nine-day drug regime (CatS 1E11 + 5Fu (IC50))
9 in xenograft model organs.

10

11 Fig 10 illustrates immunohistochemical detection of
12 CatS expression in 5-Fu-treated tumour sections.
13 Sections were stained with Mouse IgG1 isotype
14 control or CatS 1E11 MAb.

15

16 Fig 11. Immunohistochemical detection of CatS
17 expression in untreated and 5-Fu-treated (15mg/kg)
18 tumour sections. Sections were stained with CatS
19 1E11 mAb.

20

21 Fig 12 illustrates immunohistochemical detection of
22 CatS expression in untreated and CPT-11-treated
23 (15mg/kg) tumour sections. Sections were stained
24 with CatS 1E11 mAb.

25

26 Fig 13 illustrates immunohistochemical detection of
27 CatS expression in untreated and 5-Fu-treated tumour
28 sections. Sections were stained with Mouse IgG
29 Isotype control (R&D Systems) or CatS 1E11 mAb.

30

31 Fig 14 illustrates apoptosis Detection in control
32 (Mouse IgG1 + 5-Fu) and treated (CatS 1E11 + 5Fu

1 (IC50) tumour sections. Sections were stained with
2 anti-cleaved caspase-3 (Cell Signalling Technology).
3

4 Figure 15a shows photographs of HMEC cells cultured
5 in vehicle only control, an isotype control, an anti
6 Cathepsin S antibody (mAb1), or an anti Cathepsin S
7 antibody (mAb2);
8

9 Figure 15b illustrates graphs illustrating the
10 inhibition of capillary cell branching observed in
11 the presence of 1E11 (upper panel) or 1E4 (lower
12 panel).
13

14 Figure 16a illustrates photographs of sections of
15 aorta cultured in the presence of a control antibody
16 and anti Cathepsin S antibody 1E11 at 60, 300 and
17 600nM concentrations.
18

19 Figure 16b (top left) illustrates graph summarising
20 the effect of the antibody on vessel length as shown
21 in Figure 16a;
22

23 Figure 16b (top right) illustrates a graph
24 summarising the effect of the antibody on vessel
25 number as shown in Figure 16a;
26

27 Figure 16b (bottom) illustrates a graph summarising
28 the effect of the antibody on maximum vessel length
29 as shown in Figure 16a;
30

31 Figure 17a(1) and a(2) illustrates photographs of
32 sections of aorta cultured in the presence of a

1 control antibody and anti Cathepsin S antibody 1E11
2 at 1ug/ml, 5ug/ml, 10ug/ml, and 100ug/ml, 1ug/ml
3 concentrations at x4 and x20 magnification
4 respectively.

5

6 Figure 17b illustrates a graph summarising the
7 effect of the antibody in the experiment illustrated
8 in Figure 17a on number of tubules, mean tubule
9 length and maximum tubule length.

10

11 Figure 18 illustrates the amino acid sequence of the
12 V_H and V_L chains of the 1E11 inhibitory antibody,
13 with CDRs highlighted in bold and underlined, as
14 determined from DNA sequencing of the V_H and V_L
15 regions.

16

17 Materials and Methods.

18

19 Cell lines and culture conditions

20

21 The HCT116 (p53 wild type) human colorectal
22 adenocarcinoma cell line was maintained in McCoy's
23 medium (Invitrogen, UK). The RKO (p53 wild type),
24 H630 (p53 mutant) and HT29 (p53 mutant) colorectal
25 adenocarcinoma cell lines were maintained in
26 Dulbecco's Modified Eagle's Medium (DMEM,
27 Invitrogen, UK). All medium was supplemented with
28 10% FCS, 1% pen / strep, 1% L-Glutamine (All
29 Invitrogen, UK).

30

31 Treatment of cell lines with chemotherapeutic agents

32

1 Cells in a log phase of growth were seeded into 6
2 well tissue culture plates at ~20% confluence and
3 incubated overnight to allow adherence to the plate.
4 Wells were treated with CPT11 (Irinotecan) and 5-Fu
5 (Flourouracil) at varying concentrations (including
6 7.5µM) for 48 hours. Chemotherapy was substituted
7 with saline in control wells.

8

9 Western Blotting

10

11 Cells were washed in PBS (5 minute centrifugation at
12 1500rpm) prior to lysis with RIPA buffer containing
13 a protease inhibitor cocktail (Calbiochem, UK) as
14 previously described. Whole cell lysates were loaded
15 onto 12% SDS-PAGE gels at equal concentrations. Gels
16 were run overnight at 50V prior to semi-dry transfer
17 onto nitrocellulose membrane at 20V for 45 minutes
18 (BioRad, UK). The nitrocellulose membrane was
19 blocked using PBS (3% Milk powder) for ~1 hour, and
20 washed x 3 with PBS (0.01% tween) for 5 minutes. The
21 membrane was then probed with a 1 in 1000 dilution
22 of CatS Mab (1E4 Mab or 1E11 Mab) in PBS (3% Milk
23 powder) for 1 hour. The membrane was washed x 3 with
24 PBS (0.01% tween) for 5 minutes prior to the
25 addition of a 1 in 5000 dilution of secondary goat
26 anti mouse-HRP conjugated antibody (BioRad, UK) in
27 PBS (3% Milk powder) for 1 hour. This was then
28 washed three times in PBS (0.01% tween) for 5
29 minutes each. The membrane was 'developed' by ECL
30 (enhanced chemi-luminescence) using the Super Signal
31 kit (Pierce). The membrane was incubated in ECL
32 solution for 5 minutes, prior to exposure to

1 photographic film for various time periods. The
2 photographic film was washed in developer solution
3 (Sigma, UK) for 1 minute, rinsed with water, and
4 washed in fixer solution (Sigma, UK) again for 1
5 minute before being rinsed and dried.

6

7 Flow Cytometry

8 Cells were collected from tissue culture plates
9 using a scraper and washed x 3 in PBS (0.01% tween)
10 (5 minute centrifugation at 1500rpm). Approximately
11 5×10^5 cells were placed in appropriately labelled
12 tubes. Cells were incubated with 50 μ l anti-CatS or
13 Isotype control (Sigma, UK) for 1 hour at room
14 temperature. Following incubation each tube was
15 washed x 3 in PBS (0.01% tween) (5 minute
16 centrifugation at 1500rpm). Samples were then
17 incubated with a 1 in 15 dilution of FITC-conjugated
18 goat anti-mouse secondary antibody (Sigma, UK)
19 (samples kept in dark at room temperature).
20 Following incubation each tube was washed x 3 in PBS
21 (0.01% tween) (5 minute centrifugation at 1500rpm).
22 Samples were resuspended in 0.5ml of PBS prior to
23 immediate analysis using the Cyan flow cytometer
24 (Dako, UK).

25 RT - PCR

26 RT-PCR was performed using a PTC 225 Gradient Cyclor
27 (MJ Research Incorporated). RNA was collected using
28 the RNA STAT-60 reagent (Tel-Test Friendswood, USA)

1 according to the manufacturers instructions and cDNA
2 synthesised using 1ug RNA and a reverse
3 transcriptase kit (GIBCO Invitrogen, Paisley, UK).
4 The primer sets used for RT-PCR were CatS forward
5 primer 5'- ACT CAG AAT GTG AAT CAT GGT G-3' and CatS
6 reverse primer 5'-TTC TTG CCA TCC GAA TAT ATC C-3'
7 and Beta 3 integrin primer 5'-CCTACATGACCGAAAATACCT-
8 3' and 5'-AATCCCTCCCCACAAATACTG-3' Gene expression
9 was analysed using a biomix PCR mixture (Bioline,
10 UK) containing 25ul Biomix; 1.5ul forward primer;
11 1.5ul reverse primer; 2ul cDNA; 20ul dH₂O. PCR
12 conditions consisted of an initial denaturation step
13 of 95C for 10 minutes, followed by either 32 or 45
14 cycles of 95°C for 30 sec; 55°C for 30 sec; 72°C for
15 90 sec, with a final extension of 72°C for 10
16 minutes. 5 µl of amplified product from the 32 and
17 45 cycle reactions was loaded onto a 1.5% agarose
18 gel (0.001% ethidium bromide) which was run at 90V
19 for 40 minutes prior to analysis on a UV box.

20 Xenograft Models

21 6-8 week old female SCID mice were implanted with 2
22 x 10⁶ HCT116+/+ human colorectal adenocarcinoma
23 cells into each flank. HCT116 cells in a log phase
24 of growth were harvested, washed in PBS and
25 resuspended in HBSS. They were mixed with equal
26 volumes of matrigel to give a final concentration of
27 5 x 10⁶ cells / ml. Mice were randomly separated
28 into treatment groups on day 5 after implantation
29 and treated with various regimes of CatS Mab or
30 Isotype control Mab (10mg/kg) with/without 5Fu

1 (15mg/kg daily or 70mg/kg twice weekly). All drugs
2 were administered through a bolus injection. Animals
3 were sacrificed at various time points and tumours
4 were removed for analysis.

5 Immunohistochemistry

6
7 Paraffin embedding of formalin-fixed xenograft /
8 organ tissue samples, section cutting and H&E
9 (haematoxylin and eosin) staining of tissue sections
10 were performed as previously described.

11 Immunostaining was performed using the avidin-
12 horseradish peroxidase method (Vectorlabs ABC Kit).

13
14 Briefly, sections were deparaffinised by passing
15 from xylene to alcohol to running water. Endogenous
16 peroxidase activity was blocked by incubation in 3%
17 H₂O₂ in methanol for 10 min. Sections were then
18 boiled in citrate buffer, pH6.0, for 22 min.
19 Incubation in 5% normal horse serum blocking
20 solution was carried out for 20 min at room temp.
21 Sections were incubated with primary antibody at 4°C
22 overnight. Appropriate isotype controls were used at
23 the same dilutions as primary antibodies.

24
25 For peroxidase staining, sections were incubated
26 with biotinylated pan-specific secondary antibody
27 (Vector Laboratories) for 30 min at room temp
28 followed by incubation with the Vectastain Elite ABC
29 reagent (Vector Laboratories) for a further 30 min
30 at room temp. For visualisation sections were
31 stained with 3,3'-diaminobenzidine and
32 counterstained with Gill's II hematoxylin solution.

1 Staining using the cleaved caspase-3 polyclonal
2 antibody (Cell Signalling Technology) was carried
3 out according to the manufacturer's instructions.
4 For detection of antibody localisation, after prior
5 administration to xenograft models, no primary
6 antibody incubation step was required.

7
8 AccuMax™ Array slides were stained according to the
9 manufacturers instructions using a 1:100 dilution of
10 CatS monoclonal antibody and a secondary
11 biotinylated horse antibody (Vector Laboratories,
12 CA). Slides were counter stained with haematoxylin
13 prior to mounting and analysis.

14 15 Confocal Microscopy

16 HCT116 cells were stained for CatS expression using
17 a CatS monoclonal antibody as before. Cells were
18 grown on glass coverslips, fixed using ice-cold
19 acetone and incubated with CatS antibody at a 1:100
20 dilution. Coverslips were rinsed with PBS and
21 incubated with antimouse AlexaFluor® 488 labelled
22 secondary antibody. Coverslips were washed again
23 using PBS, mounted using PermaFluor mounting media
24 and visualised by confocal microscopy.

25

26 **Capillary-Like Tube Formation Assay**

27

28 The effect of the CatS mAb on endothelial cell tube
29 formation was assessed as follows. Two hundred
30 microliter of Matrigel (10 mg/ml) was applied to
31 pre-cooled 48-well plates, incubated for 10 min at
32 4°C and then allowed to polymerize for 1 h at 37°C.

1 Cells were suspended in endothelial growth cell
2 medium MV (Promocell), containing 200 nM of the
3 appropriate antibody. Five hundred microliter
4 (1×10^5 cells) were added to each well. As
5 controls, cells were incubated with vehicle-only
6 control medium containing the appropriate volumes of
7 PBS. After 24 h incubation at 37°C and 5% CO₂, cells
8 were viewed using a Nikon Eclipse TE300 microscope.
9

10 **Cell viability and proliferation assays**

11
12 Cytotoxic and proliferative effects of the CatS
13 monoclonal antibody on U251mg astrocytoma cells can
14 be tested as previously described. Briefly, cells
15 are added to a final concentration of 1×10^4
16 cells/200 μ l per well of a 96-well microtiter plate
17 (Corning Costar). Appropriate concentrations of
18 monoclonal antibody (100 nM) or vehicle-only control
19 media are added. Plates are incubated at 37°C and 5%
20 CO₂ for 24, 48, 72 and 96 hrs respectively. After
21 incubation, 10 μ l of 10 mg/ml MTT is added and
22 incubated for a further 2 h at 37°C and 5% CO₂. The
23 medium is carefully removed and formazan crystals
24 dissolved in 100 μ l/well of DMSO. Absorbance is
25 measured as described above and the results
26 expressed as the percentage of cell viability and
27 proliferation relative to each vehicle-only control.
28 All tests are performed in quadruplicate.
29

30 **Wound Assay**

31

1 The *in vitro* migration assay used in these studies
2 is a modified version of the method described by
3 Ashton et al (1999). HMEC-1 is plated into
4 individual chambers on a glass slide and grown to
5 90% confluence overnight. The medium is removed and
6 the monolayer wounded. The monolayer is re-
7 supplemented with fresh medium and the required
8 volume of antibodies added to give the required
9 final concentration.

10
11 Slides are removed at fixed time points until
12 complete closure of the wound, then fixed in 4% PBS
13 buffered paraformaldehyde. The extent of "wound"
14 closure is blindly assessed microscopically by an
15 independent investigator and quantified using a
16 calibrated eyepiece graticule (1mm/100µm graduation)
17 at 20x magnification (Olympus BX 50). The extent of
18 closure in the antibody treated slides is compared
19 to time matched sham treated controls and the %
20 inhibition of wound closure compared to time matched
21 controls calculated.

22

23 **Rat Aorta Model**

24

25 Male Wistar rats are euthanised and the thoracic
26 aorta is aseptically removed and sectioned into 1 cm
27 thick rings. The rings are washed ten times in
28 sterile medium to remove any bacteria and embedded
29 into Matrigel on 24 well plates. The wells are
30 supplemented with 2ml of medium and increasing
31 concentrations of antibodies. The plate is incubated
32 for 8 days and post incubation the Matrigel and

1 rings are fixed in 4% PBS buffered paraformaldehyde
2 and stored in PBS. The extent of vessel development
3 is blindly assessed microscopically by an
4 independent investigator and quantified using a
5 calibrated eyepiece graticule (1mm/100µm graduation)
6 at 20x magnification (Olympus BX 50). The extent of
7 vessel length, maximum vessel length and number of
8 vessels in each field of view is measured and
9 compared to time matched sham controls and the %
10 inhibition calculated.

11

12 **Sponge Assay**

13

14 Polyether sponges are subcutaneously implanted in
15 C57 black mice and injected on alternate days with
16 10 ng bFGF or 10 ng bFGF+ antibodies. After 14 days
17 of treatment, sponges are harvested, sectioned and
18 stained with H&E (A).

19

20 **Oxygen-induced retinopathy (OIR) neonatal mouse** 21 **model**

22

23 The animal model of oxygen-induced retinopathy
24 employed in this investigation is that described by
25 Smith et al and now widely used for studies testing
26 anti-angiogenic therapies for retinal
27 neovascularisation (Smith et al., 1994). Litters of
28 postnatal mice and their nursing dams are placed in
29 75% oxygen at postnatal day 7 (P7) and returned to
30 room air at P12. During the 5-day period of
31 hyperoxia the central retinal capillary beds undergo
32 vascular regression and halted development. Upon

1 return to room air the central retina becomes
2 measurably hypoxic with an associated increase in
3 vasogenic growth factors of the VEGF family. An
4 aggressive neovascular response ensues with extra-
5 retinal new vessels developing by P15.
6 Neovascularization is maximal at P17 and regresses
7 after P21.

8
9 Unfixed neural retina are dissected and subjected to
10 ultrasonic disruption in RIPA buffer. For
11 quantification of HP-protein binding, retinal
12 samples from P13 and P17 mice are prepared for a HP
13 competitive enzyme-linked immunosorbent assay
14 (ELISA) methodology. Briefly, 100 µl of sample or
15 standard in duplicate are serially diluted in PBS/5%
16 Tween 20 in a 96-well polystyrene assay plate
17 (Corning Inc., Corning, NY) and incubated with 25 µl
18 of rabbit polyclonal antibody against HP (1:3300 in
19 PBS/5% Tween) for 1 hour at 37°C. The contents of
20 each well is then transferred to NUNC C96 Maxisorp
21 plates that are precoated with solid phase antigen
22 (1:5000 in carbonate buffer, pH 9.6) overnight at
23 4°C and blocked with 1% gelatin for 1 hour. The
24 competition between solid phase and soluble antigens
25 proceeds for 1 hour at 37°C, after which the wells
26 are washed 4 x 5minutes with PBS/5% Tween, and 100
27 µl of 1:2000 alkaline-phosphatase goat anti-rabbit
28 IgG (Sigma-Aldrich) is added to each well. Plates
29 are then washed 4 x 5minutes with PBS/5% Tween, and
30 100 µl of 1 mg/ml of the alkaline-phosphatase
31 substrate, 4-nitrophenyl phosphate disodium salt
32 hexahydrate (Sigma-Aldrich) dissolved in 10%

1 diethanolamine buffer (pH 9.6) is added to each
2 well. The color development of the subsequent
3 reaction is measured at 405 nm every 5 minutes for 2
4 hours on a Safire microplate reader (Tecan
5 Instruments) and reaction kinetics analyzed using
6 Magellan 3 software. Sample HP-1 binding is
7 determined by comparison to a Lineweaver-Burk enzyme
8 kinetic standard curve, and is expressed as a
9 proportion of sample protein concentration. The
10 solid-phase antigen used in the assay is HP, which
11 is reductively bound to bovine serum albumin.
12 Statistical analysis is conducted using the
13 Student's t-test and a P value ≤ 0.05 is considered
14 significant.

15

16 **Results**

17

18 This study investigated the expression of CatS on
19 colorectal cancer cell lines and the ability of
20 chemotherapy to induce CatS expression. It further
21 examined the effects of an Anti CatS specific
22 monoclonal antibody on tumour development and
23 viability both *In Vitro* and *In Vivo*.

24

25 **Examples 1 to 3**

26 CatS expression was examined at the mRNA, protein
27 and cell surface levels.

28 Example 1: RT semi quantitative PCR was used to
29 examine mRNA levels of CatS in four colorectal cell
30 lines (HCT116 +/+, RKO +/+, H630 p53 mutant and HT29
31 p53 mutant) with/without 7.5 μ M CPT11 treatment. RNA
32 levels were analysed following 32 and 45 cycle of

1 PCR to determine relative differences in expression
2 between treated and untreated samples. All four cell
3 lines displayed basal expression of CatS while both
4 the HCT116+/+ and RKO+/+ cell lines showed an
5 increase in expression in response to chemotherapy
6 as shown in Figure 1.

7
8 **Example 2:** CatS expression was examined at the
9 protein level by performing western blot analysis on
10 whole cell lysate preparations made from three cell
11 lines (HCT116 +/+, RKO +/+ and HT29). Whole cell
12 lysates were prepared on samples with / without 48
13 hour CPT11 treatment (7.5µM) for both the HCT116+/+
14 and RKO+/+ cell lines. All three cell lines showed
15 positive expression of CatS (Figure 2) with no
16 obvious difference in whole protein expression
17 levels evident between the treated and untreated
18 HCT116+/+ or RKO+/+ samples.

19
20 **Example 3:** Cell surface expression of CatS was
21 examined using flow Cytometry. HCT116 +/+, RKO +/+,
22 and H630 p53 mutant cells were analysed for cell
23 surface expression with / without CPT11 treatment.
24 The RKO +/+ cell line showed 38% positive basal cell
25 surface expression which was increased to 49%
26 following chemotherapy induction (Figure 3 A-B). The
27 HCT116 +/+ cell line showed 39% positive basal cell
28 surface expression which was increased to 59%
29 following chemotherapy induction (Figure 3 C-D). The
30 H630 p53 mutant cell line showed 37% positive basal
31 cell surface expression which was increased to 41%
32 following chemotherapy induction (Figure 4 A-B).

1

2 **Example 4**

3 The presence of Cathepsin S on the cell surface of
4 tumour cells was further confirmed using confocal
5 microscopy. HCT116 cells were stained for CatS
6 expression using a CatS monoclonal antibody as
7 before. The results are shown in Figure 5.

8 Immunocytochemical analysis using the 1E11 CatS
9 monoclonal antibody, revealed that CatS expression
10 is localised to the cell surface on HCT116 cells,
11 which was also confirmed by flow cytometry analysis,
12 where a 38% increase in fluorescent intensity was
13 observed in HCT116 cells stained for CatS in
14 comparison to the isotype control

15

16 **Example 5**

17 In contrast, normal sections of colon do not show
18 the expression of Cathepsin S on the cell surface as
19 demonstrated for colorectal carcinomas. Figure 6
20 shows the results of immunohistochemical detection
21 of CatS expression in colorectal carcinoma biopsies.
22 Tissue sections were stained for CatS expression
23 using a monoclonal antibody raised against the
24 mature human CatS protease. Staining of normal
25 sections of human colon demonstrated little or no
26 staining for CatS, whereas colorectal carcinoma
27 biopsy sections had dense staining for CatS.

28

29 **Example 6**

30 It has been reported that Cathepsin S co-localises
31 with integrin $\alpha\beta 3$ as a receptor on the vascular
32 smooth muscle cell surface in atherosclerosis and

1 restenosis after angioplasty (Cheng et al, Am J
2 Pathology, 2006, 168:685-694).

3

4 The present inventors investigated whether the
5 localisation of Cathepsin S on the surface of tumour
6 cells demonstrated herein was associated with such
7 integrins. As shown, in Figure 7, which shows the
8 results of analysis of CatS and $\beta 3$ integrin RNA
9 expression in HCT116 +/+, colorectal cancer cells.
10 following 35 cycles of PCR, although Cathepsin S is
11 strongly expressed, no $\beta 3$ integrin RNA expression
12 was demonstrated, showing that the localisation of
13 Cathepsin S on the cell surface of tumour cells is
14 unrelated to its expression in vascular smooth
15 muscle cells under conditions of atherosclerosis.

16

17 **Example 7**

18 Studies were then carried out on the effect of
19 combination therapy using the chemotherapeutic agent
20 5Fu and the Cathepsin S inhibitory antibody 1E11
21 (also referred to herein as CatS Mab).

22

23 As shown in co-pending PCT application
24 WO2006/109045, the contents of which are
25 incorporated herein by reference, which claims
26 priority from GB0507219.4 and GB0507272.3, this
27 antibody has potent inhibitory activity. In a
28 fluorimetric assay using the fluorogenic substrate,
29 the antibody inhibits the proteolytic activity of
30 CatS. Further the antibody has also been shown to
31 potently inhibit tumour cell invasion in invasion
32 assays using the PC3 prostate carcinoma cell line,

1 the U251mg astrocytoma cell line, the HCT116
2 colorectal carcinoma cell line, the MDMB231 breast
3 cancer cell line and the MCF 7 breast cancer cell
4 line. Furthermore, antiangiogenesis effects of this
5 antibody have been demonstrated in a Matrigel assay,
6 in which the antibody was shown to inhibit capillary
7 tubule formation (see later).

8

9 Studies into *in vivo* expression of CatS were carried
10 out in Xenograft models of colorectal cancer. Models
11 were treated with the chemotherapeutic agents 5Fu,
12 CPT-11 and oxaliplatin (15mg/kg-70mg/kg doses) alone
13 or in combination with 10mg/kg 1E11 CatS monoclonal
14 antibody. Paraffin embedded tissue sections were
15 examined for antibody localisation following
16 treatment. Tumour samples obtained from animals
17 treated with CatS Mab in combination with 5-Fu
18 stained positive for IgG (Figures 8a and 8b) showing
19 that the CatS Mab can be successfully directed to
20 the positively expressing tumour cells.

21

22 **Example 8**

23 All major mouse organs were examined for
24 architectural changes following treatment. No signs
25 of pathological change were seen. Fig 9 shows
26 photographs of gut and liver sections after nine-day
27 drug regime (CatS 1E11 + 5Fu (IC50)) in xenograft
28 model organs. The *in vivo* studies suggest that
29 repeated treatment with 10mg/kg of 1E11 Mab in
30 combination with chemotherapy has no adverse toxic
31 effect (Figure 9).

32

1 Example 9

2 Tumour sections were stained using the 1E11 mAb to
3 detect CatS expression (Fig 10). A mouse IgG isotype
4 was used as a control. Figures 11 and 13 show that,
5 in tumour sections obtained from mice treated with
6 5-Fu an upregulation in CatS expression was observed
7 with Figure 12 showing a similar result in sections
8 obtained from mice treated with CPT-11.

9

10 Example 10

11 The effect of combination treatment on tumour tissue
12 was examined using an anti-cleaved caspase-3
13 polyclonal antibody (Figure 14). An increase in
14 caspase 3 activity was observed in treated tissue
15 indicating that the CatS Mab is having an influence
16 on apoptosis.

17

**18 Example 11 CatS Antibodies can inhibit tube-like
19 formation in human endothelial cells**

20

21 Using the Matrigel morphogenesis assay described by
22 Grant et al (Cell 1989 Sep 8;58(5):933-43.),
23 capillary-tubule formation assays were performed
24 with human microvascular endothelial cells (HMECs)
25 cultured on Matrigel enabling the endothelial cells
26 form tube-like structures, with invasive sprouts
27 extending from individual cells to form contacts
28 with nearby endothelial cells. Figure 15a
29 illustrates the results when the HMEC cells were
30 cultured in the presence of two CatS antibodies
31 (1E11 and 1E4) or isotype control. Extensive tube-
32 like structures are evident in the vehicle-only

1 control and isotype control antibody (200 nM)
2 panels; however this tube formation is almost
3 completely abolished in the
4 presence of either of the 1E11 (mAb1) or 1E4 (mAb2)
5 antibodies (200 nM). These results were then
6 quantified as shown in Figure 15b, which illustrates
7 the inhibition of capillary cell branching observed
8 in the presence of 1E11 (upper panel) or 1E4 (lower
9 panel).

10

11 Both antibodies have been previously shown to bind
12 specifically to CatS with no cross-reactivity with
13 other cathepsins, in particular those with the
14 greatest homology to CatS. Antibodies 1E11 and 1E4
15 have both been characterised for their ability to
16 inhibit the catalytic activity of CatS; 1E11 can
17 specifically inhibit the activity of CatS whereas
18 1E4 has no discernable effect. Therefore, the
19 results from the capillary-tube assay show that the
20 sequestering of active CatS secreted from the
21 endothelial cells by either an inhibitory or non-
22 inhibitory CatS mAb is sufficient to prevent the
23 migration and arrangement of the endothelial cells
24 into tube-like structures.

25

26 **Example 12 CatS antibodies can inhibit tube like**
27 **formation in rat aortic arch ex vivo model**

28

29 To evaluate further the role of CatS in
30 angiogenesis, an ex vivo rat aortic arch assay was
31 performed. Sections of the aorta (1 mm) were
32 cultured within a thin layer of Matrigel in the

1 presence of the inhibitory antibody and appropriate
2 controls. The formation of tube-like vessels from
3 the aorta were monitored and quantified after 7 days
4 by measuring the reduction in the number of vessels,
5 mean vessel length and maximum vessel length
6 compared to controls.

7
8 Figure 16 illustrates the significant inhibition of
9 tube formation in the presence of the CatS 1E11
10 antibody. Photographs of the ring segments are shown
11 in Figure 16a with the results summarised in Figure
12 16b. Incubation of the rat aortic ring segments with
13 up to 600nM of 1E11 resulted in greater than 80%
14 reduction in total vessel number, mean vessel length
15 and maximum vessel length (figure 16b).

16
17 Figure 17 illustrates the significant inhibition of
18 tube formation in a repeat experiment in the
19 presence of the CatS 1E11 antibody. Photographs of
20 the ring segments are shown in Figure 17a with the
21 results summarised in Figure 17b. Incubation of the
22 rat aortic ring segments with up to 10 µg/ml of 1E11
23 resulted in a 70% reduction in total vessel number
24 and a 60% reduction in both mean vessel length and
25 maximum vessel length.

26
27 All documents referred to in this specification are
28 herein incorporated by reference. Various
29 modifications and variations to the described
30 embodiments of the inventions will be apparent to
31 those skilled in the art without departing from the
32 scope and spirit of the invention. Although the

1 invention has been described in connection with
2 specific preferred embodiments, it should be
3 understood that the invention as claimed should not
4 be unduly limited to such specific embodiments.
5 Indeed, various modifications of the described modes
6 of carrying out the invention which are obvious to
7 those skilled in the art are intended to be covered
8 by the present invention.

9

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11

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28

1 Claims

2

3

4 1. A method of inhibiting chemotherapy
5 induced upregulation of Cathepsin S on
6 the surface of tumour cells, said method
7 comprising the administration of a
8 Cathepsin S inhibitor to said cells.

9

10 2. A method of treating neoplastic disease
11 comprising the simultaneous, sequential
12 or separate administration of a Cathepsin
13 S inhibitor and a chemotherapeutic agent.

14

15 3. The method according to claim 2, wherein
16 the chemotherapeutic agent is a platinum
17 based antineoplastic agent, an
18 antimetabolite, a nucleoside analog, a
19 thymidylate synthase inhibitor or a
20 topoisomerase inhibitor.

21

22 4. The method according to claim 3, wherein
23 the chemotherapeutic agent is CPT-11.

24

25 5. The method according to claim 3, wherein
26 the chemotherapeutic agent is 5-FU.

27

28 6. The method according to any one of the
29 preceding claims wherein the Cathepsin S
30 inhibitor is a small molecule inhibitor,
31 a peptide inhibitor, an antibody
32 molecule, or a nucleic acid encoding a

- 1 peptide Cathepsin S inhibitor or
2 antibody molecule Cathepsin S inhibitor.
3
- 4 7. The method according to claim 6, wherein
5 the Cathepsin S inhibitor is an antibody
6 molecule.
7
- 8 8. The method according to claim 7, wherein
9 the antibody molecule and the
10 chemotherapeutic agent are provided as an
11 immunoconjugate.
12
- 13 9. The method according to claim 8, wherein
14 the antibody molecule is cleavable from
15 the chemotherapeutic agent by protease
16 activity.
17
- 18 10. The method according to claim 7, wherein
19 the antibody molecule and the
20 chemotherapeutic agent are separate
21 molecules.
22
- 23 11. The method according to any one of claims
24 7 to 10, wherein the antibody molecule
25 binds Cathepsin S and inhibits its
26 proteolytic activity.
27
- 28 12. The method according to any one of claims
29 7 to 11, wherein the antibody molecule
30 comprises an antigen binding domain
31 comprising (i) at least one of the CDRs
32 with an amino acid sequence selected from

1 the group consisting of Seq ID No: 1, Seq
2 ID No: 2, Seq ID No: 3, or a variant
3 thereof in which 5 or less, for example
4 4, 3, 2, or 1 amino acid substitutions
5 have been made in at least one CDR,
6 and/or (ii) at least one of the CDRs
7 with an amino acid sequence consisting of
8 Seq ID No: 4, Seq ID No: 5 and Seq ID No:
9 6, or a variant thereof, in which 5 or
10 less, for example 4, 3, 2, or 1 amino
11 acid substitutions have been made in at
12 least one CDR; wherein the antibody
13 molecule retains the ability to inhibit
14 the proteolytic activity of Cathepsin S.
15

16 13. The method according to any one of claims
17 7 to 12, wherein the antibody molecule
18 comprises an antigen binding domain
19 comprising at least two of the CDRs with
20 an amino acid sequence selected from the
21 group consisting of Seq ID No: 1, Seq ID
22 No: 2 and Seq ID No: 3, and/or at least
23 two of the CDRs with an amino acid
24 sequence consisting of Seq ID No: 4, Seq
25 ID No: 5 and Seq ID No: 6.
26

27 14. The method according to any one of claims
28 7 to 13, wherein the antibody molecule
29 comprises a CDR having the amino acid
30 sequence SEQ ID NO: 5 and/or the CDR
31 having the amino acid sequence SEQ ID NO:

- 1 6.
2
3 15. The method according to any one of claims
4 7 to 14, wherein the antibody molecule
5 comprises an antibody V_H domain and an
6 antibody V_L domain, wherein the antibody
7 V_H domain comprises at least one of the
8 CDRs with an amino acid sequence selected
9 from the group consisting of Seq ID No:
10 1, Seq ID No: 2, Seq ID No: 3 and the
11 antibody V_L domain comprises at least one
12 of the CDRs with an amino acid sequence
13 consisting of Seq ID No: 4, Seq ID No: 5
14 and Seq ID No: 6.
15
16 16. The method according to any one of claims
17 7 to 15, wherein the antibody V_H domain
18 comprises CDRs with amino acid sequences
19 Seq ID No: 1, Seq ID No: 2 and Seq ID No:
20 3 as CDRs 1, 2 and 3 respectively.
21
22 17. The method according to any one of claims
23 7 to 16, wherein the antibody V_H domain
24 comprises the amino acid sequence Seq ID
25 No: 7.
26
27 18. The method according to any one of claims
28 7 to 147, wherein the antibody V_L domain
29 comprises CDRs with amino acid sequences
30 Seq ID No: 4, Seq ID No: 5 and Seq ID No:
31 6 as CDRs 1, 2 and 3 respectively.
32

- 1 19. The method according to any one of claims
2 7 to 18, wherein the antibody V_L domain
3 comprises the amino acid sequence Seq ID
4 No: 8.
5
6
7 20. The use of a Cathepsin S inhibitor and a
8 chemotherapeutic agent in the preparation
9 of a medicament for combination therapy
10 by simultaneous, sequential or separate
11 administration of the Cathepsin S
12 inhibitor and chemotherapeutic agent in
13 the treatment of a neoplastic disease.
14
15 21. A pharmaceutical composition comprising a
16 Cathepsin S inhibitor and a
17 chemotherapeutic agent.
18
19 22. A pharmaceutical kit comprising a
20 Cathepsin S inhibitor and a
21 chemotherapeutic agent for combination
22 therapy by simultaneous, sequential or
23 separate administration of the Cathepsin
24 S inhibitor and chemotherapeutic agent.
25
26 23. The use of Cathepsin S as a biomarker for
27 tumour cells.
28
29 24. A method of inhibiting expression of
30 Cathepsin S on the surface of tumour
31 cells, said method comprising the
32 administration of a Cathepsin S

1 inhibitor.

2

3 25. A method of inhibiting chemotherapy
4 induced upregulation of Cathepsin S on
5 the surface of tumour cells, said method
6 comprising the administration of a
7 Cathepsin S inhibitor to said cells.

8

9 26. A method of inducing antibody dependent
10 cell-mediated cytotoxic reaction against
11 a tumour cell, said method comprising the
12 administration of an anti-Cathepsin S
13 antibody molecule to said cell, wherein
14 the antibody or fragment thereof does not
15 inhibit the proteolytic activity of
16 Cathepsin S.

17

18 27. A method of killing tumour cells, said
19 method comprising the administration of
20 an anti-Cathepsin S antibody molecule to
21 said cells, wherein the antibody or
22 fragment thereof does not inhibit the
23 proteolytic activity of Cathepsin S.

24

25 28. A method of treating neoplastic disease
26 in a subject, said method comprising the
27 administration of an anti-Cathepsin S
28 antibody molecule to said subject wherein
29 the antibody or fragment thereof does not
30 inhibit the proteolytic activity of
31 Cathepsin S.

32

1 29. The use of an anti-Cathepsin S antibody
2 or a fragment thereof which binds
3 Cathepsin S in the preparation of a
4 medicament for the treatment of a
5 neoplastic disease, wherein the antibody
6 or fragment thereof does not inhibit the
7 proteolytic activity of Cathepsin S.

8
9 30. The method according to any one of claims
10 26 to 28 or the use according to claim
11 29, wherein the anti-Cathepsin S
12 antibody or fragment thereof is
13 administered in the absence of another
14 chemotherapeutic agent

15
16 31. A method of inhibiting angiogenesis in a
17 group of cells, a tissue or an organ,
18 said method comprising administration of
19 an antibody molecule or a nucleic acid
20 encoding said antibody molecule to said
21 cells, tissue or organ, wherein said
22 antibody molecule specifically binds
23 Cathepsin S but does not inhibit the
24 proteolytic activity of Cathepsin S.

25
26 32. A method of treating a condition
27 associated with activity of Cathepsin S
28 in a patient in need of treatment
29 thereof, said method comprising
30 administration of an antibody molecule or
31 a nucleic acid encoding said antibody
32 molecule to said patient, wherein said

1 antibody molecule specifically binds
2 Cathepsin S but does not inhibit the
3 proteolytic activity of Cathepsin S.
4

5 33. The use of an antibody molecule in the
6 preparation of a medicament for the
7 treatment of a condition associated with
8 aberrant activity of Cathepsin S,
9 antibody molecule or a nucleic acid
10 encoding said antibody molecule, wherein
11 said antibody molecule specifically
12 binds Cathepsin S but does not inhibit
13 the proteolytic activity of Cathepsin S.
14

15 34. The method according to claim 32 or the
16 use according to claim 33, wherein said
17 condition is associated with aberrant
18 expression of Cathepsin S.
19

20 35. The method according to claim 32 or claim
21 34, or the use according to claim 33 or
22 34 wherein said condition is associated
23 with angiogenesis.
24

25 36. The method or use according to claim 35,
26 wherein said disease is cancer.
27

28 37. The method or use according to claim 35,
29 wherein said disease is an inflammatory
30 condition.
31

- 1 38. The method or use according to claim 35,
2 wherein said disease is an ocular
3 disease.
4
- 5 39. The method or use according to claim 35,
6 wherein said disease is atherosclerosis.
7
- 8 40. An antibody molecule or a nucleic acid
9 encoding said antibody molecule for use
10 in medicine, wherein said antibody
11 molecule specifically binds Cathepsin S
12 but does not inhibit the proteolytic
13 activity of Cathepsin S
14
- 15 41. A pharmaceutical composition comprising
16 an antibody molecule or a nucleic acid
17 encoding said antibody molecule, wherein
18 said antibody molecule specifically binds
19 Cathepsin S, but does not inhibit the
20 proteolytic activity of Cathepsin S.
21
- 22 42. The pharmaceutical composition according
23 to claim 41, wherein said composition
24 further comprises an inhibitor of VEGF.
25
- 26 43. The pharmaceutical composition according
27 to claim 41, wherein said inhibitor is an
28 anti VEGF antibody.
29
- 30 44. The pharmaceutical composition according
31 to claim 41, wherein said composition
32 further comprises an inhibitor of EGF

1 receptor.

2

3 45. The pharmaceutical composition according
4 to claim 44, wherein said inhibitor is an
5 anti-EGF receptor antibody.

6

7 46. A method of identifying tumour cells in a
8 population of cells, said method
9 comprising bringing into contact a ligand
10 with binding specificity for Cathepsin S
11 with said population of cells and
12 determining the presence or absence of
13 binding of the ligand to cells in said
14 population, wherein binding is indicative
15 of the presence of tumour cells within
16 said population of cells.

17

18 47. The method according to claim 46, wherein
19 the method is performed in vitro or ex
20 vivo.

21

22 48. A method for evaluating in vitro the
23 response of tumour cells from a subject
24 to the presence of a chemotherapeutic
25 agent to predict response of the tumour
26 cells in vivo to treatment with the
27 chemotherapeutic agent which method
28 comprises : (a) providing an in vitro
29 sample from a subject containing tumour
30 cells; (b) exposing a portion of said
31 sample of tumour cells to said
32 chemotherapeutic agent; (c) comparing

1 expression of Cathepsin S on the surface
2 of said cells with expression of
3 Cathepsin S on the surface of cells in a
4 control portion of said sample which has
5 not been exposed to said chemotherapeutic
6 agent; wherein enhanced expression in the
7 portion of sample exposed to said
8 chemotherapeutic agent is indicative of
9 sensitivity to said chemotherapeutic
10 agent.

11
12 49. The method according to claim 48, wherein
13 enhanced expression in the portion of
14 sample exposed to said chemotherapeutic
15 agent is indicative of suitability of the
16 patient for treatment with a Cathepsin S
17 inhibitor in addition to said
18 chemotherapeutic agent.
19
20
21
22