

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  $\beta$  peptide (A $\beta$ ) 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  $\alpha v \beta 3$  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  $\alpha$ -keto- $\beta$ -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**

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

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

In a first aspect, there is provided the use of a Cathepsin S inhibitor, wherein the Cathepsin S inhibitor is an antibody molecule or a nucleic acid encoding said antibody molecule, and a chemotherapeutic agent in the preparation of a medicament for combination therapy by simultaneous, sequential or separate administration of the Cathepsin S inhibitor and chemotherapeutic agent in the treatment of a neoplastic disease.

In a second aspect, there is provided a pharmaceutical composition comprising a Cathepsin S inhibitor and a chemotherapeutic agent, wherein said Cathepsin S inhibitor is an anti-Cathepsin S antibody molecule.

In a third aspect, there is provided a pharmaceutical kit comprising a Cathepsin S inhibitor and a chemotherapeutic agent for combination therapy by simultaneous, sequential or separate administration of the Cathepsin S inhibitor and chemotherapeutic agent, wherein said Cathepsin S inhibitor is an anti-Cathepsin S antibody molecule.

In a fourth aspect, there is provided an anti-Cathepsin S antibody molecule for use in inducing antibody dependent cell-mediated cytotoxic reaction against a tumour cell, wherein the antibody or fragment thereof does not inhibit the proteolytic activity of Cathepsin S.

In a fifth aspect, there is provided an anti-Cathepsin S antibody for use in killing tumour cells, wherein the antibody or fragment thereof does not inhibit the proteolytic activity of Cathepsin S.

In one embodiment of the present disclosure, there is provided a method of identifying a tumour cell in a population of cells, said method comprising bringing into contact a ligand with binding specificity for Cathepsin S with said population of cells and determining the presence or absence of binding of the ligand to cells in said population, wherein binding is indicative of the presence of tumour cells within said population of cells.

The above embodiment may be performed in vitro. In another embodiment, the method is performed in vivo.

The disclosure also describes employment of the cell-surface CatS as a biomarker for the use of antibody delivered drug conjugates. This strategy could be further exploited by the delivery of pro-drugs to the cells, which are activated specifically by the enzymatic activity of CatS.

In a further embodiment of the present disclosure, there is provided the use of Cathepsin S as a biomarker for tumour cells.

Moreover, the present inventors have found that treatment of tumour derived cell lines with 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 embodiment of the present disclosure,  
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 embodiment of the disclosure 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 embodiment, the disclosure 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 embodiment of the present disclosure, there is  
8 provided a pharmaceutical composition comprising a  
9 Cathepsin S inhibitor and a chemotherapeutic agent.

10

11 Also described herein is that 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  
19 described herein may therefore find use in  
20 treatment regimes involving any class of chemotherapeutic  
21 agent. For example, chemotherapeutic agents, which may be  
22 used include  
23 platinum based antineoplastics, antimetabolites,  
24 nucleoside analogs, thymidylate synthase inhibitors,  
25 ortopoisomerase inhibitors.

26

27 In one example, 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.  
10 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  $\alpha$ -keto- $\beta$ -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, described herein is  
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  
10 the Cathepsin S inhibitor is an antibody molecule, which  
11 binds Cathepsin S and inhibits its proteolytic activity.  
12 Any antibody molecule capable of inhibiting the proteolytic  
13 effect of Cathepsin S may be used in such aspects.

14

15

16 In an embodiment, 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<sub>H</sub> domain or an antibody V<sub>L</sub> domain, or  
12 both.

13

14 In one embodiment, the antibody molecule has an  
15 antibody V<sub>H</sub> 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<sub>L</sub> 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<sub>L</sub> domain  
25 comprises the amino acid sequence Seq ID No: 8 and  
26 /or the antibody V<sub>H</sub> domain comprises the amino acid  
27 sequence Seq ID No: 7.

28

29 Seq ID No: 7:

30 VQLQESGGVLVKPGGSLKLSAASGFAFSSYDMSWVRQTPEKRLEWVAYITT  
31 GGVNTYYPDITVKGRFTISRDNKNTLYLQMSLKS EDTAMYYCARHSYFDY  
32 WGQGT TVTVSS

33

1 Seq ID No: 8:

2 DVLMTQTPLSLPVS LGDQASISCRSSQSLVHSNGNTYLHWYLQKPGQSPKLL  
3 IYKVS NRFS GVPDR FSGSGSGTDFTLKISRVEAEDLGVYFCSQTTHVPPTFG  
4 SGT KLEIKR  
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  
14 herein 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, the antibody molecule for use herein  
28 has the ability to inhibit tumour cell invasion.  
29

30  
31 In another embodiment, the antibody molecule for  
32 use herein has the ability to inhibit angiogenesis.  
33

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 embodiment described herein,  
17 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 further embodiment, 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 above methods of the seventh and eighth embodiment of  
2 the invention may be performed in vitro, in vivo or ex  
3 vivo, as required.

4

5 Accordingly, in a ninth embodiment of the present  
6 disclosure, 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 a tenth embodiment, the disclosure 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 any one of the seventh to tenth embodiment, the  
24 antibody  
25 molecule thereof does not inhibit the proteolytic  
26 activity of Cathepsin S.

27

28

29 In any one of the seventh to tenth embodiment, 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 embodiment  
2 described herein.  
3

4 As described above, in the methods described herein,  
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 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 embodiment, the present disclosure  
32 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 embodiment of the disclosure,  
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 embodiments 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 embodiment, 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 provides as a further embodiment 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 embodiments described herein  
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 methods described herein 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 embodiment of the present  
21 disclosure, any suitable anti-Cathepsin S antibody molecule, which  
22 does not inhibit the proteolytic activity of Cathepsin S, but which  
23 inhibits angiogenesis, may be used.

24

25

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

29

30 In one example of the fifteenth embodiment 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, the antibody molecule of and for  
6 use herein has the ability to inhibit tumour cell invasion.  
7

8

9 According to a sixteenth embodiment of the disclosure,  
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 herein as a seventeenth embodiment  
17 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 embodiment of the disclosure 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 embodiment described herein  
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  $\alpha$ -keto- $\beta$ -  
14 aldehydes. The dipeptide  $\alpha$ -keto- $\beta$ -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')<sub>2</sub>, 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')<sub>2</sub> 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](http://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: 3<sup>rd</sup> 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 induces G<sub>2</sub>-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 ( $\text{CH}_2\text{THF}$ ) 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  $\text{CH}_2\text{THF}$  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, 21<sup>st</sup> 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 (methylmethacrylate) 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, 21<sup>st</sup> 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 ( $S_e$ /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 ( $S_{obs}$ ) 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 Romanelli (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 tested. 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  $1 \times 10^4$  cells/200  $\mu$ 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% CO<sub>2</sub> for 24, 48,  
31 72 and 96 hrs respectively. After incubation, 10 $\mu$ l  
32 of 10 mg/ml MTT is added and incubated for a further

1 2 h at 37°C and 5% CO<sub>2</sub>. 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  $\mu$ 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  $\mu$ 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  $\mu$ 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  $\mu$ 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<sub>H</sub> and V<sub>L</sub> chains of the 1E11 inhibitory antibody,  
13 with CDRs highlighted in bold and underlined, as  
14 determined from DNA sequencing of the V<sub>H</sub> and V<sub>L</sub>  
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 McCoys  
23 medium (Invitrogen, UK). The RKO (p53 will 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 7.5 $\mu$ M) 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  $5 \times 10^5$  cells were placed in appropriately labelled  
12 tubes. Cells were incubated with 50 $\mu$ 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 Cycler  
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 dH<sub>2</sub>O. 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 ul 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  $\times 10^6$  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  $5 \times 10^6$  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 H<sub>2</sub>O<sub>2</sub> 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 ( $1 \times 10^5$  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% CO<sub>2</sub>, 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  $1 \times 10^4$   
16 cells/200  $\mu$ 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 CO<sub>2</sub> for 24, 48, 72 and 96 hrs respectively. After  
21 incubation, 10 $\mu$ l of 10 mg/ml MTT is added and  
22 incubated for a further 2 h at 37°C and 5% CO<sub>2</sub>. The  
23 medium is carefully removed and formazan crystals  
24 dissolved in 100  $\mu$ 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  $\mu$ l 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  $\mu$ l  
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  $\mu$ l 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  $\mu$ l 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  $\leq 0.05$  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 $\mu$ 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  $\alpha v \beta 3$  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           1.    Use of a Cathepsin S inhibitor, wherein  
3                the Cathepsin S inhibitor is an antibody  
4                molecule or a nucleic acid encoding said  
5                antibody molecule, and a chemotherapeutic  
6                agent in the preparation of a medicament  
7                for combination therapy by simultaneous,  
8                sequential or separate administration of  
9                the Cathepsin S inhibitor and  
10               chemotherapy agent in the treatment  
11               of a neoplastic disease.
- 12
- 13           2.    The use according to claim 1, wherein the  
14                chemotherapeutic agent is a platinum  
15                based antineoplastic agent, an  
16                antimetabolite, a nucleoside analog, a  
17                thymidylate synthase inhibitor or a  
18                topoisomerase inhibitor.
- 19
- 20           3.    The use according to claim 2, wherein the  
21                chemotherapeutic agent is CPT-11.
- 22
- 23           4.    The use according to claim 2, wherein the  
24                chemotherapeutic agent is 5-FU.
- 25
- 26
- 27
- 28
- 29
- 30
- 31
- 32
- 33
- 34

- 1           5.    The use according to any one of the  
2               preceding claims, wherein the antibody  
3               molecule and the chemotherapeutic agent  
4               are provided as an immunoconjugate.  
5
- 6           6.    The use according to claim 5, wherein the  
7               antibody molecule is cleavable from the  
8               chemotherapeutic agent by protease  
9               activity.  
10
- 11          7.    The use according to any one of the  
12               claims 1 to 4, wherein the antibody  
13               molecule and the chemotherapeutic agent  
14               are separate molecules.  
15
- 16          8.    The use according to any one of the  
17               preceding claims, wherein the antibody  
18               molecule binds Cathepsin S and inhibits  
19               its proteolytic activity.  
20
- 21          9.    The use according to any one of the  
22               preceding claims, wherein the antibody  
23               molecule comprises an antigen binding  
24               domain comprising (i) at least one of the  
25               CDRs with an amino acid sequence selected  
26               from  
27  
28  
29  
30  
31  
32  
33  
34

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

27 11. The use according to any one of the  
28 preceding claims, wherein the antibody  
29 molecule comprises a CDR having the amino  
30 acid sequence SEQ ID NO: 5 and/or the CDR  
31 having the amino acid sequence SEQ ID NO:  
32  
33  
34

- 1 6.
- 2
- 3 12. The use according to any one of the
- 4 preceding claims, wherein the antibody
- 5 molecule comprises an antibody V<sub>H</sub> domain
- 6 and an antibody V<sub>L</sub> domain, wherein the
- 7 antibody V<sub>H</sub> domain comprises at least one
- 8 of the CDRs with an amino acid sequence
- 9 selected from the group consisting of Seq
- 10 ID No: 1, Seq ID No: 2, Seq ID No: 3 and
- 11 the antibody V<sub>L</sub> domain comprises at least
- 12 one of the CDRs with an amino acid
- 13 sequence consisting of Seq ID No: 4, Seq
- 14 ID No: 5 and Seq ID No: 6.
- 15
- 16 13. The use according to any one of the
- 17 preceding claims, wherein the antibody V<sub>H</sub>
- 18 domain comprises CDRs with amino acid
- 19 sequences Seq ID No: 1, Seq ID No: 2 and
- 20 Seq ID No: 3 as CDRs 1, 2 and 3
- 21 respectively.
- 22
- 23 14. The use according to any one of the
- 24 preceding claims, wherein the antibody V<sub>H</sub>
- 25 domain comprises the amino acid sequence
- 26 Seq ID No: 7.
- 27
- 28 15. The use according to any one of the
- 29 preceding claims, wherein the antibody V<sub>L</sub>
- 30 domain comprises CDRs with amino acid
- 31 sequences Seq ID No: 4, Seq ID No: 5 and
- 32 Seq ID No: 6 as CDRs 1, 2 and 3
- 33 respectively.
- 34

- 1           16. The use according to any one of the  
2           preceding claims, wherein the antibody V<sub>L</sub>  
3           domain comprises the amino acid sequence  
4           Seq ID No: 8.  
5
- 6           17. A pharmaceutical composition comprising a  
7           Cathepsin S inhibitor and a  
8           chemotherapeutic agent, wherein said  
9           Cathepsin S inhibitor is an anti-  
10          Cathepsin S antibody molecule.  
11
- 12          18. A pharmaceutical kit comprising a  
13          Cathepsin S inhibitor and a  
14          chemotherapeutic agent for combination  
15          therapy by simultaneous, sequential or  
16          separate administration of the Cathepsin  
17          S inhibitor and chemotherapeutic agent,  
18          wherein said Cathepsin S inhibitor is an  
19          anti-Cathepsin S antibody molecule.  
20
- 21          19. The use of Cathepsin S as a biomarker for  
22          colorectal tumour cells.  
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1           20.    An anti-Cathepsin S antibody molecule  
2                   for use in inducing antibody dependent  
3                   cell-mediated cytotoxic reaction against  
4                   a tumour cell, wherein the antibody or  
5                   fragment thereof does not inhibit the  
6                   proteolytic activity of Cathepsin S.

7  
8           21.    An anti-Cathepsin S antibody for use in  
9                   killing tumour cells, wherein the  
10                  antibody or fragment thereof does not  
11                  inhibit the proteolytic activity of  
12                  Cathepsin S.

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- 1           22. 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           23. The anti-Cathepsin S antibody molecule  
10          according to any one of claims 20 to 21  
11          or the use according to claim 22, wherein  
12          the anti-Cathepsin S antibody or fragment  
13          thereof is administered in the absence of  
14          another chemotherapeutic agent  
15  
16          24. An antibody molecule or a nucleic acid  
17          encoding said antibody molecule for use  
18          in inhibiting angiogenesis, wherein said  
19          antibody molecule specifically binds  
20          Cathepsin S but does not inhibit the  
21          proteolytic activity of Cathepsin S.  
22  
23          25. An antibody molecule or a nucleic acid  
24          encoding said antibody molecule for use  
25          in treating a condition associated with  
26          activity of Cathepsin S in a patient in  
27          need of treatment thereof, wherein said  
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1 antibody molecule specifically binds  
2 Cathepsin S but does not inhibit the  
3 proteolytic activity of Cathepsin S.  
4  
5 26. 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 27. The antibody molecule or nucleic acid  
16 according to claim 25, wherein said  
17 condition is associated with aberrant  
18 expression of Cathepsin S.  
19  
20 28. The antibody molecule or nucleic acid  
21 according to claim 25 or claim 27, or the  
22 use according to claim 26 wherein said  
23 condition is associated with  
24 angiogenesis.  
25  
26 29. The antibody molecule or nucleic acid or  
27 use according to claim 28, wherein said  
28 disease is cancer.  
29  
30 30. The antibody molecule or nucleic acid or  
31 use according to claim 28, wherein said  
32 disease is an inflammatory condition.  
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- 1           31. The antibody molecule or nucleic acid or  
2           use according to claim 28, wherein said  
3           disease is an ocular disease.  
4  
5           32. The antibody molecule or nucleic acid or  
6           use according to claim 28, wherein said  
7           disease is atherosclerosis.  
8  
9           33. An antibody molecule or a nucleic acid  
10          encoding said antibody molecule for use  
11          in medicine, wherein said antibody  
12          molecule specifically binds Cathepsin S  
13          but does not inhibit the proteolytic  
14          activity of Cathepsin S  
15  
16          34. A pharmaceutical composition comprising  
17          an antibody molecule or a nucleic acid  
18          encoding said antibody molecule, wherein  
19          said antibody molecule specifically binds  
20          Cathepsin S, but does not inhibit the  
21          proteolytic activity of Cathepsin S.  
22  
23          35. The pharmaceutical composition according  
24          to claim 34, wherein said composition  
25          further comprises an inhibitor of VEGF.  
26  
27          36. The pharmaceutical composition according  
28          to claim 35, wherein said inhibitor is an  
29          anti VEGF antibody.  
30  
31          37. The pharmaceutical composition according  
32          to claim 34, wherein said composition  
33          further comprises an inhibitor of EGF  
34  
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- 1                   receptor.
- 2
- 3           38. The pharmaceutical composition according
- 4           to claim 37, wherein said inhibitor is an
- 5           anti-EGF receptor antibody.
- 6
- 7           39. A method of identifying colorectal tumour
- 8           cells in a cell sample, said method
- 9           comprising bringing into contact a ligand
- 10          with binding specificity for Cathepsin S
- 11          with said cell sample and determining the
- 12          presence or absence of binding of the
- 13          ligand to cells in said cell sample,
- 14          wherein binding is indicative of the
- 15          presence of colorectal tumour cells
- 16          within said cell sample.
- 17
- 18          40. The method according to claim 39, wherein
- 19          the method is performed in vitro or ex
- 20          vivo.
- 21
- 22          41. 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
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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 42. The method according to claim 41, 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.

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