GREMLIN-1 ANTAGONIST FOR THE PREVENTION AND TREATMENT OF CANCER

Field of the Invention

The present invention relates to an anti-GREM1 antagonist for use in a method of treatment of cancer. The cancer is typically a solid cancer having a stroma, typically having stromal GREM1 overexpression. The cancer may have epithelial GREM1 overexpression. The present invention further relates to combination therapy with a GREM1 antagonist and an additional anti-cancer (such as chemotherapeutic) agent, and related compositions. The present invention also relates to detection, prognosis and selections of treatment for cancer based on stromal GREM1 overexpression.

Background to the Invention

Gremlin-1 (also known as Drm and CKTSF1B1 and GREM1) is a 184 amino acid glycoprotein which forms part of the DAN family of cystine-knot secreted proteins (along with Cerberus and Dan amongst others). GREM1 binds and inhibits the ability of BMP-2, 4, and 7 to signal along with a documented pro-angiogenic role possibly through agonism of VEGFR2. The main role of GREM1 is during development, in which it is vital during kidney formation and during limb bud formation. These vital roles make GREM1 homozygous knock-outs embryonic lethal in mice.

In adulthood, increased levels of GREM1 have been associated with idiopathic pulmonary fibrosis and pulmonary arterial hypertension in which BMP2, 4 and 7 signalling is reduced with an associated rise in TGFβ levels. In both diabetic and chronic allograft nephropathy, GREM1 expression has been correlated with fibrosis score.

Increased levels of GREM1 have also been associated *inter alia* with scleroderma, diabetic nephropathy, glioma, head and neck cancer, prostate cancer and colorectal cancer (Sneddon et al; Guan et al). GREM1 has been shown to activate cancer cell invasion and proliferation *in vitro* and is thought to play a role in uterine, cervix, lung, ovary, kidney, breast, colon, pancreatic and sarcoma carcinomas.

There is a need to identify effective therapies for use in treatment and prevention of cancer.

Summary of the Invention

The inventors have surprisingly shown that GREM1 antagonists are effective therapeutic and preventative agents against neoplasia with stromal and/or epithelial GREM1 overexpression, including colorectal cancer and multiple myeloma. It is envisaged by the inventors based on these results that GREM1 antagonists will be of general utility in treatment and prevention of cancer, including other cancers having stromal GREM1 overexpression. The *in vivo* results provided herein illustrate long-term prevention of induction of neoplasia in various mouse tumour models by administration of a GREM1 antagonist, and significant therapeutic impact on existing tumours by administration of a GREM1 antagonist. The inventors' findings thus provide for a new approach to prevention and treatment of cancer, including in cancers resistant to standard chemotherapeutic agents.

Thus, in a first aspect of the present invention there is provided an anti-GREM1 antagonist for use in a method for the treatment or prevention of a cancer.

In a further aspect of the invention, there is provided an anti-cancer agent for use in a method for the treatment of a cancer wherein the method comprises separate, sequential or simultaneous administration of an anti-GREM1 antagonist.

In another aspect of the invention, there is provided a method of treating a cancer comprising administering a therapeutically effective amount of an anti-GREM1 antagonist to a subject in need thereof.

In yet another aspect of the invention, there is provided a composition or kit comprising an anti-GREM1 antagonist and an additional anti-cancer agent.

In a further aspect of the invention, there is provided a method for detecting cancer in a patient, the method comprising measuring stromal expression of GREM1 in the patient, wherein stromal overexpression of GREM1 indicates that the patient comprises a cancer.

In yet a further aspect of the invention, there is provided a method for prognosing a cancer in a patient, the method comprising determining whether or not the cancer

comprises stromal overexpression of GREM1, wherein stromal overexpression of GREM1 in the cancer indicates that the patient has a worse prognosis than in the situation of normal stromal expression of GREM1.

In another aspect of the invention, there is provided a method for determining whether or not a patient having or suspected of having or being at risk of developing cancer is likely to respond to treatment with a chemotherapeutic agent, which method comprises measuring stromal expression of GREM1 in the patient, and thereby predicting whether or not the patient is likely to respond to treatment with the chemotherapeutic agent.

In yet another aspect of the invention, there if provided a method for determining whether or not a patient having or suspected of having or being at risk of developing cancer is likely to respond to treatment with a GREM1 antagonist, the method comprising measuring stromal expression of GREM1 in the patient, and thereby predicting whether or not the patient is likely to respond to treatment with the GREM1 antagonist.

Brief Description of the Figures

Figure 1. Percentage restoration of signal for the immunisation derived antibodies in the HEK-ID1 reporter gene assay.

Figure 2. Percentage restoration of signal for library derived antibodies in the HEK-ID1 reporter gene assay.

Figure 3. Results for the HEK-ID1 reporter gene assay with titrations of human Gremlin (Figure 3A) and mouse Gremlin (Figure 3B) and the effect of antibody 7326 (shown as antibody PB376) in restoring signalling of BMP.

Figure 4. A structural model of the Gremlin-Fab complex, with the possible BMP binding regions and the Fab epitope highlighted.

Figure 5. Organoid culture from mouse intestinal crypts at day 0 and day 7 following seeding. Media contains recombinant protein supplementation and/or test anti-Grem1 antibody. E=Epidermal growth factor, G=Grem1, R=Rspo1.

Figure 6. Western blot on proteins extracted from Vil1-Grem1 mouse epithelium. 6 weeks of antibody administration at 30mg/kg is able to restore epithelial pSMAD1,5 signalling.

Figure 7. Antibody treated *Vil1-Grem1* animal phenotype after 6 weeks of treatment with anti-Grem1 antibody. Antibody treatment normalises small intestinal phenotype and prevents profound polyposis development. Antibody treatment prevents villus ectopic crypt formation and normalises cell fate determination with appropriate restriction of Ki67, Sox9, EphB2 and lysozyme staining to the base of the intestinal crypt, and resolution of normal CK20 staining in the differentiating cells of the small intestinal villus.

Figure 8. Kaplan-Meier plots of survival of Grem1 initiated polyposis mouse strains following long term anti-Grem1 antibody treatment.

Figure 9. Stromal Grem1 in Apc-driven tumourigenesis. A. Grem1 ISH on wildtype and Villin-CreERT2; Apc fl/fl mice showing profound upregulation of stromal Grem1 in response to acute epithelial Apc loss. B. Transgenic deletion of stromal Grem1 in ApcMin mice reduces mutant Apc tumour burden at 285 days. C. Prolonged treatment with anti-Grem1 antibody (30mg/kg/week) reduces ApcMin tumour burden and prolongs animal lifespan.

Figure 10. Grem1 expression is elevated in primary stromal cultures from myeloma patients. RNA was extracted from bone marrow trephine derived stromal cell cultures from age and gender matched healthy donors and myeloma patients and the expression on Grem1 was analysed by real-time PCR. The expression of Grem1 was significantly higher in the myeloma patient cohort comparative to healthy donors. Data presented as mRNA expression normalised to ActB. (mean ± SD, **P<0.001, T-test, Normal; n=17 and MM; n=15).

Figure 11. Grem1 expression was (A) analysed in the compact bone of healthy C57Bl6/KaLwRij.Hsd mice and mice injected with 5TGM1.Bmx1 MM PCs with disease detectable via BLI. (mean ± SD, P=0.1120, T-test, Normal; n=11 and Tumour-bearing; n=9). (B) Grem1 expression in the BM stroma isolated from the hind limbs of tumour-bearing mice was correlated with the tumour burden in the respective limbs, as detected by BLI (Pearson Correlation; $p<0.05$, $R2=0.414$).

Figure 12. Grem1 expression was analysed in the murine, bone-marrow derived stromal OP9 cell lines following 24, 48 and 72 hr co-culture with (A) 5TGM1.Bmx1 cells, co-cultured in the upper 3µm transwell (B) 5TGM1.parental cells, plated directly onto the adherent stromal cells. For contact culture, OP9.GFP+ cells were sorted by FACS from the 5TGM1.parental MM PCs for analysis of Grem1 expression via real-time PCR. A significant increase in Grem1 expression was observed in the OP9 stromal cells following 72hrs of co-culture. Data presented as mRNA expression relative to ActB and normalised to media only control. (mean ± SEM of 3 replicate experiments, *$P<0.05$, t-test).

Figure 13. Gremlin1 expression was analysed in normal human stroma co-cultured with various human MM cell lines for 72hrs. MM cells were washed from stroma prior to collection for analysis of Grem1 expression. Significant increase in Gremlin1 expression in KMS-11 ($p=0.0159$) and U266 ($p=0.0343$) co-culture conditions (ANOVA). Data presented as replicates from co-culture with three separate normal stroma donors, normalised to media only control.

Figure 14. Grem1 transgene expression in OP9-stromal cells was confirmed by **(A)** RT-PCR and **(B)** Western blot. 5TGM1 MM PCs in **(C)** cell contact **(D)** transwell co-cultures with OP9-stromal cells modified to overexpress Grem1 displayed increased rates of proliferation compared to co-culture with OP9 vector-only controls, as measured by relative luciferase activity. (Mean ± SEM of 3 replicate experiments, ** $P<0.01$, ***$P<0.001$, t-test).

Figure 15. (A) C57Bl6/KaLwRij mice injected with 5TGM1.Bmx1 MM PCs and subsequently treated with a Grem1-neutralising antibody displayed a significant reduction

in overall tumour burden compared to mice treated with an IgG control after 4 weeks as shown by BLI. Mean ± SEM, n=13 mice per group ****P< 0.0001, one-way ANOVA. (B) SPEP was performed on mice bled at week 4 post-tumour cell injection. M-spike intensity relative to serum albumin was used a measure of disease burden. Mice treated with the anti-Grem1 antibody had significantly lower M-spike intensity compared to mice receiving the IgG control treatment. Mean ±SD, **P<0.01, t-test. (C) Representative BLI ventral scan images for IgG control and Grem1-neutralising antibody treated mice.

Figure 16. 5TGM1 MM cells were co-cultured in 3μm transwells in the presence of OP9-Grem1 overexpressing cells or vector-only controls for 72hrs. Lysate from the 5TGM1 cells was analysed by Western blot for phosphorylation of Smads 1/5/9. 5TGM1 cells display a reduction in phosphorylation of Smads 1/5/9 when cultured in the presence of Grem1-overexpressing BM stromal cells, compared to vector-only controls. Hsp90 was used as a loading control. Image representative of two replicate experiments.

Figure 17. The BM stromal cell line, OP9 was assessed for expression of Grem1 following treatment with 20ng/ml of recombinant IL6 for 72hrs. A significant increase in Grem1 expression was observed in the OP9 cells stimulated with IL6. (Mean ± SEM of 3 replicate experiments, ** P<0.01).

Figure 18. (A) C57Bl6/KaLwRij mice that received treatment of Grem1-neutralising antibody (Ab7326) prior to inoculation with 5TGM1.Bmx1 MM PCs displayed a significant reduction in overall tumour burden at 4 weeks post-cell inoculation, compared to mice treated with that received an IgG control (Ab101.4), as shown by BLI. Mean ± SEM, n=7-8 mice per group ****P< 0.0001, one-way ANOVA. (B) SPEP was performed on mice bled at week 4 post-tumour cell injection. M-spike intensity relative to serum albumin was used a measure of disease burden. Mice treated with the anti-Grem1 antibody had significantly lower M-spike intensity compared to mice receiving the IgG control treatment. Mean ±SD, **P<0.0001, t-test. (C) Representative BLI ventral scan images for IgG control and Grem1-neutralising antibody treated mice 4-weeks post-tumour cell inoculation.

Figure 19. Quantitative RT-PCR analysis of Grem1 mRNA expression. Grem1 gene expression was analysed in (A) human MDA-MB-231-TXSA breast cancer cells and (B) human MF9 mammary fibroblasts following 48hr culture in normoxic and hypoxic conditions (n=1). Expression levels were measured by RT-PCR and normalised to β-actin. Mean ± SD of triplicate wells, *p<0.05, **p<0.005, Student's unpaired t-test.

Figure 20. Human breast cancer cell proliferation in response to stimulation with Grem1. MDA-MB-231-TXSA cells were stimulated with various concentrations of rhGrem1 and cultured under normoxic (left-hand bar of each pair) and hypoxic conditions (right-hand bar of each pair) for (A) 24 and (B) 48 hrs. Data is a representative of three independent experiments performed in triplicate. Mean ± SD, *p<0.05, **p<0.005, ***p<0.0005, two-way ANOVA with Tukey's multiple comparisons test.

Figure 21. Murine 4T1 breast cancer cell proliferation in response to stromal-derived Grem1. 4T1 cells were co-cultured with Grem1-expressing or vector control OP9 stromal cells for 72hrs. Data is a representative of three independent experiments performed in triplicate. Mean ± SD, ***p<0.0005, Student's unpaired t-test (two-sided).

Figure 22. Novel anti-Grem1 antibody (Ab7326) reverses Grem1-mediated inhibition of Smad1/5/9 phosphorylation in murine 4T-1 breast cancer cells. Murine 4T1 breast cancer cells were stimulated for 2hrs with combinations of treatments as indicated and subjected to Western blot analysis using an antibody reactive to phospho-Smad1/5/9 (upper panel). β-actin (lower panel) served as a loading control.

Figure 23. The effect of monotherapy and combination therapy in VG/Min mice.

Figure 24. Grem1 expression in various solid tumours.

Figure 25. The relationship between Grem1 expression and survival implications in bladder carcinoma. Top line – Grem1 low; bottom line – Grem1 high.

Figure 26. The relationship between Grem1 expression and survival implications in pancreatic ductal adenocarcinoma. Top line – Grem1 low; bottom line – Grem1 high.

Figure 27. The relationship between Grem1 expression and survival implications in ovarian adenocarcinoma. Top line – Grem1 low; bottom line – Grem1 high.

Figure 28. The relationship between Grem1 expression and survival implications in basal breast carcinoma. Top line – Grem1 low; bottom line – Grem1 high.

Figure 29. The relationship between Grem1 expression and survival implications in lung carcinoma. Top line – Grem1 low; bottom line – Grem1 high.

Figure 30. Antibody treated *Vil1-Grem1* bowel phenotype after 6 weeks of treatment with anti-Grem1 antibody.

Figure 31. Kaplan-Meier plots of survival of Grem1 initiated polyposis mouse strains following long term anti-Grem1 antibody treatment. (A) *Vil1-Grem1* mice. (B) *Vil1-Grem1;Apc^{Min}* mice.

Figure 32. (A) Kaplan-Meier plot showing that prolonged treatment with anti-Grem1 antibody (30 mg/kg/week) reduces *Apc^{Min}* tumour burden and prolongs animal lifespan. (B) *In situ* hybridization for *Apc^{Min}* and *Grem1*.

Figure 33. Tumour burden in C57Bl6/KaLwRij mice is significantly reduced in the hindlimb bones when mice are treated with Grem1-neutralising antibody, Ab7326, (A) post-tumour cell inoculation and (B) pre-tumour cell inoculation, compared to IgG control (as shown by bioluminescent imaging (BLI)). A downwards trend in splenic tumour burden is observed between treatment groups in the (C) post-, or (D) pre-tumour cell inoculation setting. Students t-test, ** $p< 0.01$, *** $p< 0.001$.

Figure 34. Kaplan Meier survival plots for treatment of established polyposis in (A) *Vil1-Grem1* mice and (B) *Apc^{Min}* mice. Median survival: *Apc^{Min}* Vehicle: 192 days (n=15);

Apc^{Min} anti-Grem1: 264.5 days (n=10); *Vil-Grem1* Vehicle: 242 days (n=13); and *Vil-Grem1 anti-Grem1:* 477 days (n=8), log rank p<0.01 in both cases. Arrows indicate treatment initiation in treated groups.

Figure 35. Plots showing ssurvival and colonic polyp burden in four treatment groups, namely: (i) untreated *Vil1-Grem1* mice (VG + no treatment); (ii) *Vil1-Grem1* mice treated with azoxymethane only (VG + AOM + no treatment); (iii) *Vil1-Grem1* mice treated with anti-Grem1 antibody only (VG + α-*Grem1*); and (iv) *Vil1-Grem1* mice treated with both azoxymethane and anti-Grem1 antibody (VG + AOM + α-*Grem1*). (A) Kaplan-Meier curve denoting survival of mice. (B) Plot showing colonic polyp burden. (C). Plot showing colonic polyp size.

Figure 36. Stromal levels of *Foxl1* expression in villi from WT, untreated *Vil1-Grem1* and anti-Grem1 antibody treated *Vil1-Grem1* mice (*: p-value < 0.05, **: p-value <0.01).

Figure 37. Multiplex Fluorescent ISH for *Foxl1* (green) and *Wnt5a* (red) in *Vil1-Grem1* mouse small bowel. Sections were counter-stained using DAPI. Combined expression of *Foxl1* and *Wnt5a* was observed (in the yellow areas).

Figure 38. Tissue expression of Wnt ligands and telocyte cell marker in wildtype, *Vil1-Grem1* and treatment models. (A) *Wnt 5A;* (B) *Wnt 2B;* and (C) *Foxl1* telocyte marker expression using ISH in wildtype and *Vil1-Grem1* mice and quantified post antibody treatment.

Figure 39. Kaplan Meier survival curves of *Vil1-Grem1;Apc*^{Min} mouse models treated with control vehicle (line D, median survival 45 days, n=13) and with anti-Grem1 antibody at 15 mg/kg weekly (line A, median survival 76.5 days, n=6), 30 mg/kg weekly (line B, median survival 108 days, n=15, ongoing experiment: two mice still alive) and 60 mg/kg weekly (line C, median survival 111 days, n=7, ongoing experiment: five mice still alive).

Figure 40. Kaplan Meier survival curves for preventative therapy and treatment of established polyposis. Arrows indicate treatment initiation time point in treated groups.

(A) *Vil1-Grem1* mice. Vehicle (solid line): median survival 242 days (n=13); anti-Grem1 therapy late-stage treatment (dotted line): 519 days, n=7; anti-Grem1 early stage treatment (dashed line): 540 days, (n=11) log rank $p<0.01$ for both groups. (B) Apc^{Min} mice. Vehicle (solid line): median survival: 192 days (n=15); anti-Grem1 late-stage treatment (dotted line): 261 days (n=11); anti-Grem1 early stage treatment (dashed line): 424.5 days, (n=10), log rank $p<0.01$ for both groups.

Figure 41. C57BL6 mice inoculated with PyMT-B01 breast cancer cells by systemic CA injection and treated with Grem1-neutralising antibody (Ab7326) had significantly reduced tumour burden compared to mice treated with isotype control antibody (Ab101.4). (A) IVIS BLI imaging of mice at the conclusion of the study (at day 13) showed significantly lower tumour burden in Ab7326-treated mice compared with Ab101.4-treated mice. Mean ± SEM, Student's t-test, Ab101.4; n=12, Ab7326; n=13, $*p<0.05$. (B) Representative BLI images at day 13.

Figure 42. PyMT-B01 tumour bearing C57BL6 mice treated with Grem1-neutralising antibody (Ab7326) had reduced lung and liver metastasis, compared to mice treated with isotype control (Ab101.4). (A) Plot showing results of *ex vivo* BLI imaging for tumour burden in the lungs of AB7326 treated mice compared to Ab101.4 treated mice. (B) Plot showing results of BLI imaging for liver tumour burden in Ab7326 and Ab101.4 treatment groups. Mean ± SEM, Student's t-test, Ab101.4; n=6, Ab7326; n=7, $*p<0.05$.

Figure 43. Plots showing results of IVIS BLI imaging of MDA-MB-231 tumour-bearing NSG mice treated with isotype control antibody (AbA33) and Grem1-neutralising antibody (UCB6114). Mean ± SEM, Student's t-test, n=8, $p>0.05$

Figure 44. Representative IVIS BLI images of MDA-MB-231 tumour-bearing mice treated with isotype control (AbA33) or Grem1-neutralising antibody (UCB6114) at day 22 post-tumour cell inoculation.

Figure 45. Plot showing results of *ex vivo* IVIS BLI imaging of the lungs of MDA-MB-231 tumour-bearing NSG mice treated with isotype control antibody (AbA33) or Grem1-neutralising antibody (UCB6114). Mean ± SEM, Student's t-test, n=8, $p>0.05$.

Figure 46. Plots showing results of BLI imaging of NSG mice inoculated with PC-3 prostate cancer cells by systemic CA injection and treated with Grem1-neutralising antibody (UCB6114) or isotype control antibody (AbA33). BLI imaging was carried out on (A) the whole body; (B) *ex vivo* liver BLI; (C) *ex vivo* hindlimb; and (D) *ex vivo* lung. Mean ± SEM, Student's t-test, n=5-7, *p<0.05, **p<0.01, ***p<0.001.

Figure 47. Representative BLI images of NSG mice with systemic PC-3 tumour burden treated with either isotype control antibody (AbA33) or Grem1-neutralising antibody (UCB6114) at the conclusion of the study (at day 25). Liver, hindlimbs and lungs were dissected and imaged *ex vivo* immediately after the final whole-body scan.

Detailed Description of the Invention

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

In addition, as used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "an inhibitor" includes two or more such inhibitors, or reference to "an oligonucleotide" includes two or more such oligonucleotide and the like.

All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

Treatment and prevention of cancer

The present invention provides an anti-GREM1 antagonist for use in a method of prevention or treatment of cancer. The cancer typically has a stroma (such as a desmoplastic stroma), typically having stromal GREM1 overexpression. The cancer may additionally or alternatively have epithelial GREM1 overexpression or be GREM1-initiated.

Cancer

The cancer may be any cancer or tumour having a stroma, typically a desmoplastic stroma. The cancer may be any cancer or tumour which is GREM1-initiated. The cancer may be any cancer where stromal and/or epithelial GREM1 overexpression is observed. The cancer or tumour may have stromal GREM1 overexpression and no epithelial GREM1 overexpression. The cancer or tumour may have epithelial GREM1 overexpression and no stromal GREM1 overexpression. In preferred embodiments, the cancer or tumour has an overexpression of GREM1 in the desmoplastic stroma.

The cancer or tumour may be a solid tumour. The solid tumour may have a desmoplastic stroma.

Particularly preferred cancers that may be treated include colorectal cancer, multiple myeloma, pancreatic cancer, bladder cancer, breast cancer, lung cancer, stomach cancer, duodenal cancer, oesophageal cancer, prostate cancer, head and neck cancer, endometrial cancer, liver cancer, spleen cancer, bone-resident cancer, and osteosarcoma. The cancer that may be treated may be intestinal cancer, colon cancer, or rectal cancer. The cancer to be treated may be a disseminated cancer, for example a metastatic cancer. A disseminated cancer should be understood as one that has spread from its initial site of origin within the body. For example, a disseminated cancer could be one that originated in the bone marrow, colon, prostate, or breast tissue of a patient but has spread to *e.g.* the patient's liver or lung.

The GREM1 antagonist may also be used to prevent the dissemination of a cancer.

The GREM 1 antagonist may be used to prevent polyposis associated with a cancer.

Grading systems are used in cancer biology and medicine to categorize cancer cells with respect to their lack of cellular differentiation. This reflects the extent to which the cancer cells differ in morphology from healthy cells found in the tissue from which the cancer cell originated. The grading system can be used to provide an indication of how quickly a particular cancer might be expected to grow. Typically used grades of cancer are Grades (G) X and 1 to 4. GX indicates that the cancer grade cannot be assessed. G1 (low grade) cancer cells have a similar morphology to normal, healthy, cells (*i.e.* they are well differentiated) and would be expected to grow slowly, and are less likely to spread. G2 (intermediate grade) cancer cells are moderately differentiated, *i.e.* they appear more abnormal and would be expected to grow slightly faster than G1 cells. G3 (high grade)

cancer cells have a very different morphology compared to normal cells (*i.e.* they are poorly differentiated) and would be expected to grow faster than G1 and G2 cells. G4 (high grade) cancer cells are undifferentiated (also referred to as anaplastic) and would be expected to have the highest capacity for proliferation.

Cancer grading is different to cancer staging, which gives an indication of how a cancer might spread. A common cancer staging system has five stages, namely Stage 0: cancer cells *in situ* (*i.e.* located in their normal tissue); Stage I: cancers are localized to one part of the body; Stage II: cancers are locally advanced; Stage III: cancers are also locally advanced (whether a cancer is designated as Stage II or Stage III can depend on the specific type of cancer); and Stage IV: cancers have often metastasized, or spread to other organs or throughout the body.

A person skilled in the art knows how to determine the grade and/or stage of a cancer. In one embodiment, the invention relates to use of an anti-GREM1 antagonist for the treatment and/or prevention of an established cancer. In one embodiment, the cancer is an established cancer. An established cancer may be a high grade cancer, for example a G3 or a G4 cancer. An established cancer may be a cancer that is Stage II or above. An established cancer may be a Stage III or a stage IV cancer. In one embodiment, the established cancer is a Stage IV cancer that has metastasized.

Colorectal cancer

The invention relates in one preferred aspect to prevention or treatment of colorectal cancer. By way of background, the intestinal mucosa is a complex ecosystem and the epithelium has an inter-dependent relationship with its microenvironment, particularly the underlying stroma. Mesenchymal-epithelial crosstalk is intimately involved in regulating homeostasis and is dynamically altered in intestinal regeneration and cancer. Cell-signalling networks are the effector pathways of inter-compartmental crosstalk and control epithelial cell fate determination, but can be co-opted and corrupted by the tumour microenvironment in colorectal cancer.

The current chemotherapeutic management of colorectal cancer has not substantially changed for the last 20 years and is predominantly based around the use of combination cytotoxic agents (such as FOLFOX and FOLFIRI regimens http://www.cancerresearchuk.org/about-cancer/cancer-in-general/treatment/cancer-

drugs/drugs) against the proliferating tumour epithelium, and resistance to these epithelial targeted agents may arise. It is now more important than ever to identify new therapies for use in colorectal cancer.

A particularly preferred cancer or tumour for treatment is thus colorectal cancer or a colorectal tumour. An especially preferred form of colorectal cancer for treatment is colorectal cancer that is characterised by having overexpression of GREM1 in stromal cells, i.e. stromal GREM1 overexpression. The stromal cells may be cancer associated fibroblasts. A colorectal cancer with stromal GREM1 overexpression may display no epithelial GREM1 overexpression. A colorectal cancer with stromal GREM1 overexpression may comprise stromal Foxl1 overexpression. A particularly suitable form of colorectal cancer for treatment is colorectal cancer that is a mesenchymal subtype colorectal cancer, also described as CMS4 (Guinney et al, Nat Med 2015). Any other subtypes of colorectal cancer may also be treated including any of CMS1, CMS2 and CMS3 as described in Guinney et al supra. A colorectal cancer as described herein may be a proximal colorectal cancer (or a proximal colorectal tumour). The proximal colon is the region of the large bowel upstream of the splenic flexure, meaning the caecum, the ascending colon and the transverse colon. Cancers or tumours in this region are also referred to as right-sided cancers or tumours. The invention may concern treating right-sided colorectal cancer or a right-sided colorectal tumour.

The colorectal cancer may be distal colorectal cancer (or a distal colorectal tumour). The distal colon is the region of the large bowel downstream of the splenic flexure, meaning the descending colon, the sigmoid colon and the rectum. Cancers or tumours in this region are also referred to as left-sided cancers or tumours. The invention may concern treating left-sided colorectal cancer or a left-sided colorectal tumour. A cancer having stromal overexpression of GREM1 may preferably be a sporadic cancer. The sporadic cancer may be caused by a somatic mutation. The sporadic cancer may be caused by a carcinogenic agent. A sporadic cancer is not due to an inherited genetic mutation. The sporadic cancer may cause the stromal overexpression of GREM1. Proliferation of the sporadic cancer may be dependent on the stromal overexpression of GREM1 in the cancer.

At least three single nucleotide polymorphisms (SNPs) close to GREM1 are independently associated with risk of colorectal cancer (CRC) in white northern

Europeans, and probably in other ethnic groups (Tomlinson et al, PLos Genet, 2011). There is a direct link with GREM1 expression and it is likely that the other SNPs have similar effects. In addition, two common SNPs near BMP2, two near BMP4 and one near BMP7 influence the expression of BMP ligands and affect CRC risk. Thus, the cancer may comprise one or more of the above SNPs.

A further type of cancer or tumour for treatment according to the invention is one that exhibits overexpression of GREM1 in epithelial cells. The overexpression of GREM1 in epithelial cells may cause the cancer. Proliferation of the cancer may be dependent on the epithelial overexpression of GREM1. Thus, the cancer may be of epithelial origin. The cancer is typically colorectal cancer or duodenal cancer. Preferably, the cancer is colorectal cancer. The cancer may be GREM1-initiated. By GREM1-initiated it is meant that a mutagenic event enhancing activity or expression of GREM1 is causative of the cancer. Such a cancer may be due to an inherited genetic mutation. The cancer may thus be a familial cancer (see below).

A preferred type of colorectal cancer for treatment may be resistant to one or more known anti-cancer agents (such as chemotherapeutic agents), as described further below.

The colorectal cancer may be a disseminated colorectal cancer. The colorectal cancer may be a metastatic colorectal cancer. The colorectal cancer may be metastatic colorectal cancer of the lung. The colorectal cancer may be metastatic colorectal cancer of the liver. The colorectal cancer may be metastatic colorectal cancer of the bone.

The colorectal cancer may be characterised by stromal overexpression of the *Foxl1*. The colorectal cancer may be characterised by stromal overexpression of one or more *Wnt* ligand. For example, the colorectal cancer may be characterised by stromal overexpression of *Wnt5A* and/or *Wnt2B*. A colorectal cancer may be particularly suitable for prevention or treatment using a GREM1 antagonist if said colorectal cancer has stromal overexpression of *Foxl1* and/or a *Wnt* ligand e.g. *Wnt5A* or *Wnt2B*.

Familial cancer

Familial cancers include cancers resulting from a mutation or mutations in the GREM1 encoding gene, or any other mutation affecting expression of the GREM1 gene. The autosomal dominant condition Hereditary Mixed Polyposis Syndrome (HMPS) is caused by a 40kb duplication upstream of GREM1 that results in a pathological

compartment expression switch from a restricted mesenchymal gradient to ectopic GREM1 gene expression throughout the epithelium.

The subject to be treated with anti-GREM1 antagonist may have been previously determined as being at risk of developing a familial cancer. For example, the subject may have been determined as being at risk on the basis of their family history and/or because the subject carries a mutation in a gene known to give rise to, or increase the risk of developing, the familial cancer.

The familial cancer may be Lynch syndrome, which is also referred to as hereditary nonpolyposis colorectal cancer (HNPCC). The familial cancer may be familial adenomatous polyposis (FAP).

The present inventors have demonstrated that use of an anti-GREM1 antibody substantially increases the survival of Apc^{Min} model mice (see *e.g.* Examples 11, 19, and 23 below). Apc^{Min} mice are a well established model for familial adenomatous polyposis (FAP). Thus, patients or subjects suffering with FAP may be particularly suitable for treatment with the anti-GREM1 antagonist. The familial cancer to be treated or prevented with the anti-GREM1 antagonist (*e.g.* an anti-GREM1 antibody) may be FAP. A subject who has previously suffered from FAP may be preventatively administered with an anti-GREM1 antagonist, *e.g.* to prevent relapse. A subject who has not previously suffered from FAP but has been previously determined to be at risk of developing FAP may be preventatively administered with an anti-GREM1 antagonist. A subject may have been determined as being at risk of developing FAP because it has been found that the subject carries a deleterious mutation in their *Apc* gene.

Multiple myeloma

The invention relates in another preferred aspect to treatment or prevention of multiple myeloma. Multiple Myeloma (MM) is a haematological malignancy characterised by the clonal proliferation of plasma cells (PCs) within the bone marrow (BM). It is well known that the BM supports tumour growth in MM, with bi-directional signalling between the tumour cells and the BM critical for the continued growth, spread and survival of the MM PCs. Cellular and non-cellular BM components exert different effects upon the growth and spread of MM PC. While recent studies have identified components of the BM that play a role in disease progression, and therapies targeting these have been developed, the

standard-of-care treatments in MM still rely primarily on targeting the tumour cells themselves. While such therapies are effective in prolonging patient survival, due to the large role the BM plays in the growth, spread, survival and drug resistance of MM cells, more effective therapies that target this important aspect of disease are needed. Indeed, MM is a largely incurable disease, with disease relapse a key issue faced in effectively treating this disease.

The invention accordingly is also preferably directed to treatment or prevention of multiple myeloma. Multiple myeloma typically comprises the presence of more than one mass of plasma cells within the bone marrow. Multiple myeloma is thus typically associated with aberrant proliferation of plasma cells in the bone marrow. An especially preferred form of multiple myeloma for treatment is characterised by having overexpression of GREM1 in the bone marrow. The multiple myeloma may therefore comprise stromal GREM1 overexpression. The stromal GREM1 overexpression may be present in the compact bone compartment of the bone. The stromal GREM1 overexpression may reflect an increased number of stromal cells, or an increase in the expression levels of GREM1 within existing GREM1-expressing stromal cells. The bone marrow may comprise osteochrondroreticular (OCR) stem cells. The stromal cells overexpressing GREM1 may comprise OCR stem cells. A preferred type of multiple myeloma for treatment may be resistant to one or more known anti-cancer agents (such as chemotherapeutic agents), as described further below.

Breast cancer

The invention relates in another preferred aspect to treatment or prevention of breast cancer. As described in more detail in the Examples, the inventors have observed that GREM1 induces breast cancer cell proliferation and further that co-culture of breast cancer cells with Grem-1 expressing stromal cells also induces such proliferation. Additionally, a GREM1 antagonist is able to neutralise activatory effects of GREM1 on breast cancer cells.

Accordingly, the results obtained in breast cancer support a role for GREM1 in breast cancer cell proliferation, including by GREM1 expression in stromal cells.

The inventors have also demonstrated the *in vivo* efficacy of a GREM1 antagonist in the prevention and treatment of breast cancer in a pre-clinical mouse models (see

Example 24 below). In particular, it was observed that breast cancer tumour-bearing mice treated with the GREM1 antagonist showed a reduction in: overall tumour burden, tumour burden in the lung, and tumour burden in the liver.

The invention therefore provides for treatment and prevention of breast cancer by administering an anti-GREM1 antagonist. The breast cancer may comprise stromal GREM1 overexpression. The stromal breast cells overexpressing GREM1 may comprise stromal fibroblasts, also described herein as cancer-associated fibroblasts. A preferred type of breast cancer for treatment may be resistant to one or more known anti-cancer agents (such as chemotherapeutic agents), as described further below. The breast cancer may be a disseminated breast cancer. The breast cancer may be a metastatic breast cancer. The breast cancer may be metastatic breast cancer of the lung. The breast cancer may be metastatic breast cancer of the liver. The breast cancer may be metastatic breast cancer of the bone.

Prostate cancer

In a further aspect, the present invention relates to the treatment or prevention of prostate cancer.

As described in more detail in Example 25, the inventors have demonstrated the efficacy of a GREM1 antagonist in the prevention and treatment of prostate cancer in a pre-clinical mouse model. In particular, it was observed that prostate cancer tumour-bearing mice treated with the GREM1 antagonist showed a reduction in: overall tumour burden, tumour burden in liver, skeletal tumour burden, and tumour burden in the lung.

The present invention therefore further provides for treatment and prevention of prostate cancer by administering an anti-GREM1 antagonist. The prostate cancer may be a disseminated prostate cancer. The prostate cancer may be a metastatic prostate cancer. The prostate cancer may be metastatic prostate cancer of the lung. The prostate cancer may be metastatic prostate cancer of the liver. The prostate cancer may be metastatic prostate cancer of the bone.

Other cancers

In addition to specifically exemplified applications in treatment and prevention of colorectal cancer, multiple myeloma, breast cancer, and prostate cancer, the inventors

envisage that the therapeutic efficacy of GREM1 antagonists as illustrated in these cancers will also be applicable to treatment of other cancers having corresponding features. In particular, it is envisaged that GREM1 antagonists will be useful in preventing or treating cancers in which there is stromal and/or epithelial GREM1 overexpression, and this overexpression contributes to malignant cell growth. Such cancers include pancreatic cancer, bladder cancer, lung cancer, stomach cancer, duodenal cancer, oesophageal cancer, head and neck cancer, glioma, endometrial cancer, liver cancer, spleen cancer, bone-resident cancer, and osteosarcoma. The relationship between Grem1 expression and survival implications in various solid tumours as determined using publically available data (from R2 server. https://hgserver1.amc.nl/cgi-bin/r2/main.cgi) is shown in Figures 24 to 29.

Stroma and epithelium

The cancers described for prevention or treatment herein may comprise stromal and/or epithelial GREM1 overexpression.

The terms "stromal cell(s)" or "stroma" as used herein refers to structural and/or connective portions of a tissue or organ.

Stromal tissue is primarily made of extracellular matrix containing connective tissue cells. Extracellular matrix is primarily composed of ground substance - a porous, hydrated gel, made mainly from proteoglycan aggregates - and connective tissue fibers. There are three types of fibers commonly found within the stroma: collagen type I, elastic, and reticular (collagen type III) fibres. Fibroblasts and pericytes are among the most common types of stromal cells.

In the context of a cancer or tumour (e.g. initiating in the epithelium of a tissue or organ), the stroma of the tissue or organ may assist cancer growth and progression. The stroma associated with the cancer or tumour may be a desmoplastic stroma caused by growth of fibrous or connective tissue around the cancer or tumour.

The overexpression of GREM1 may be observed in any part of the stroma/any stromal cells. The stromal cells may be fibroblasts or fibroblast-like support cell. The stromal cells may be fibroblasts or fibroblast-like support cell isolated from a desmoplastic stroma of any cancer or tumour described above, such as from the colon or rectum in a

colorectal cancer, or the bone marrow in multiple myeloma. The stromal cells may be cancer-associated fibroblasts.

The term "epithelial" as used herein refers to a cell derived from the outer lining of a tissue or organ. In relation to the colon, the intestinal epithelium is the layer of cells that form the luminal surface or lining of both the small and large intestine of the gastrointestinal tract. It is composed of simple columnar epithelium. The "upper barrier" is the intestinal epithelial single layer of columnar cells consisting of four intestinal epithelial cell types: the absorbent enterocytes, the goblet cells, the Paneth cells and the enteroendocrine cells. Upper barrier features are similar in small and large bowel. The main difference is constituted by the presence of elevations and projections (circular folds, villi and microvilli) in duodenum, jejunum and ileum that allows the increase of the absorption surface. This is not observed in the colon, which instead shows a flat surface. Amongst the mucous membrane protrusions termed villi, there are inflections called crypts of Lieberkühn, which are distinct glandular invaginations. The cell in which epithelial GREM1 overexpression is observed may be any epithelial cell, such as any intestinal epithelial cell.

While not being bound by theory, the present inventors postulate that overexpression of GREM1 in the epithelium and/or stroma may promote a stem/progenitor cell phenotype (increasing the number of stem/progenitor cells), promoting epithelial stem cell behaviour and driving cancer progression and/or resistance to chemotherapeutic agents. Thus, a GREM1 antagonist used according to the invention may prevent induction of an aberrant cancer stem/progenitor cell phenotype, reduce epithelial stem cell behaviour and/or decrease the number of stem/progenitor cells, in the epithelium of a tissue or organ of a subject in which cancer is to be prevented or treated, such as in the intestinal epithelium. The ability of a GREM1 antagonist to affect stem cell behaviour may be assayed clinically by the assessment of known epithelial and cancer stem cell markers.

GREM1

The terms GREM1 or Gremlin-1 as used in the present invention in the context of a protein refer to a protein that typically has the amino acid sequence as set out in the UniProt entry O60565 (SEQ ID NO: 1), human GREM1. The terms GREM1 and Gremlin-1 may also refer to a Gremlin-1 polypeptide which:

(a) comprises or consists of the amino acid sequence of SEQ ID NO: 1 with or without the N-terminal signal peptide, i.e. may comprise or consist of the mature peptide sequence as shown in SEQ ID NO: 21; or

(b) is a derivative having one or more amino acid substitutions, modifications, deletions or insertions relative to the amino acid sequence of SEQ ID NO: 1 with or without the N-terminal signal peptide (as shown in SEQ ID NO: 21), which retains the activity of Gremlin-1, such as the amino acid sequence of SEQ ID NO: 20.

(c) a variant thereof, such variants typically retain at least about 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94% or 95% identity to SEQ ID NO: 1 (or SEQ ID NO: 20 or 21) (or even about 96%, 97%, 98% or 99% identity). In other words, such variants may retain about 60% - about 99% identity to SEQ ID NO: 1, suitably about 80% - about 99% identity to SEQ ID NO: 1, more suitably about 90% - about 99% identity to SEQ ID NO: 1 and most suitably about 95% - about 99% identity to SEQ ID NO: 1. Variants are described further below.

As discussed further below, residue numbers are typically quoted based on the sequence of SEQ ID NO: 1. However, residue numbering could readily be extrapolated by the skilled person to a derivative or variant sequence as discussed above. Where residue numbers are quoted, the invention also encompasses these residues on a variant or derivative sequence.

A GREM1 or Gremlin-1 nucleic acid sequence may comprise or consist of the sequence of SEQ ID NO: 36 or SEQ ID NO: 37 or a variant thereof. Variant nucleic acid sequences are described further below. A GREM1 or Gremlin-1 nucleic sequence may comprise or consist of any GREM1 transcript variant. Examples of GREM1 transcripts variants are Transcript 1 (NCBI: NM_013372.6; ENSEMBL: ENST00000560677.5); Transcript 2: NCBI: NM_001191323.1; ENSEMBL: ENST00000560830.1); Transcript 3: NCBI: NM_001191322.1; ENSEMBL: ENST00000622074.1. The sequences available at the above accession numbers as of 18 June 2018 are incorporated by reference herein.

Overexpression

The overexpression of GREM1 in the stroma and/or epithelium may be determined by any means. The overexpression of GREM1 is typically determined by comparison to the level of the marker in normal cells of the same tissue type, i.e. basal expression level.

The expression is typically normalized against the expression level of other genes, preferably comprising one or more housekeeping genes. GREM1 may also be classified as showing an overexpression or underexpression in a threshold percentage of a population of cancer patients. The overexpression in each patient in the population may be higher than 2 from the geometric mean. At least 10%, more preferably at least 15% or more of the patients in the population may display such an overexpression.

GREM1 stromal overexpression refers to stromal GREM1 levels being higher than that of a matched normal tissue. For example, stromal GREM1 levels may be at least two fold higher than that of matched normal tissue.

Where GREM1 is overexpressed, its amount may be increased by any amount relative to basal. For example, GREM1-initiated cancers such as HMPS may comprise a several thousand-fold upregulation of epithelial GREM1, whereas no GREM1 expression is observed in normal epithelium. Sporadic cancers comprising stromal GREM overexpression may comprise any level of stromal overexpression over the physiological GREM1 expression level in normal stroma of the organ. The skilled person is able to evaluate the existence of an overexpression in stroma or epithelium compared with the level of GREM1 in normal cells of the same type.

The amount determined may be the amount of mRNA. The cancer may thus comprise an overexpression of GREM1 mRNA. The cancer may comprise an increased amount of GREM1 mRNA compared with normal cells of the same tissue type. The mRNA may be increased by any amount. The amount of mRNA can be measured using a quantitative reverse transcription polymerase chain reaction (qRT-PCR), such as real time qRT-PCR, quantigene assay (Affymetrix/Thermo Fisher), by northern blotting or using microarrays, RNA sequencing. mRNA expression is preferably determined by comparing the gene expression of a sample to the distribution of expression levels of the specific gene across a reference sample composed of tumours that are diploid for that gene. A z-score may be derived using RNAseq by expectation maximisation (RSEM) algorithm (cBioportal for Cancer Genomics, www.cbioportal.org; Gao et al, 2013 and Serami eta al 2012). A z-score of 2 SD higher or lower than the mean of the reference set is preferably considered as overexpression or underexpression respectively.

The amount determined may be the amount of protein. The cancer may comprise an overexpression of GREM1 protein, such as compared with normal cells of the same tissue

type. The protein may be increased by any amount. The amount of protein can be measured using immunohistochemistry, western blotting, mass spectrometry or fluorescence-activated cell sorting (FACS), including by use of an anti-GREM1 antibody of the invention. The thresholds for determining expression may vary between techniques used, and may be validated against immunohistochemistry scores.

The uses of GREM1 antagonists for treating or preventing cancer in a patient as described herein may thus comprise (a) measuring the amount of GREM1 in the cancer and (b) if the cancer comprises an overexpression of GREM1, administering to the patient the GREM1 antagonist and thereby treating or preventing the cancer. The amount of GREM1 may be the mRNA or protein amount, and the overexpression any overexpression discussed above.

Sample

The above measurements may be carried out in any suitable sample from the patient. The measurements may be carried out in a cancer or tumour biopsy obtained from the patient. The stroma and/or epithelium (stromal and/or epithelial cells) may be isolated from the biopsy. The biopsy tissue may be formalin fixed paraffin embedded (FFPE) tissue or fresh tissue. The tissue may be pancreatic tissue, bladder tissue, lung tissue, endometrial tissue, breast tissue, stomach tissue, duodenal tissue, oesophageal tissue, bone marrow or colorectal tissue. Any of the methods discussed above may be carried out on the cancer biopsy. Such methods may also be carried out on cancer cells circulating in the blood of the patient. The RNA methods may be carried out on urinary or blood exosomes.

Antagonist

An anti-GREM1 antagonist is any molecule that reduces the function or activity of GREM1. The anti-GREM1 antagonist may reduce function or activity of GREM1 by any amount. The anti-GREM1 antagonist may reduce GREM1 function or activity by at least 10%, at least 20%, at least 30% at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 95%, or may prevent any GREM1 function or activity. The extent to which an anti-GREM1 antagonist reduces GREM1 function or activity may be determined by measuring GREM1 function or activity in cells in the presence and absence of the anti-GREM1 antagonist. The cells may be normal cells or cancer cells. The

cells may be cancer cells as described above. They may be colorectal cancer cells. The colorectal cancer cells may be present in *Vil1-Grem1* and/or *APC(Min)* mouse models as described in the Examples. Thus, an in vivo assay for activity of a GREM1 antagonist in colorectal cancer may be performed in a mouse model of GREM1-initiated cancer or of a sporadic cancer resulting in stromal GREM1 overexpression. Alternatively, the cancer cells may be multiple myeloma cancer cells, such as plasma cells isolated from a myeloma present in the bone marrow. More generally, a GREM1 antagonist shown to reduce function or activity of GREM1 by any means may then be assayed *in vitro* or *in vivo* for its ability to prevent or reduce proliferation of cancer cells, such as colorectal cancer cells or multiple myeloma cancer cells or breast cancer cells, or to prevent, reduce or eliminate a cancer or tumour.

The antagonist may decrease GREM1 function by any means. It may increase or decrease the activity or amount of any molecule affecting GREM1 function directly or indirectly. It may decrease the amount of GREM1 at the mRNA or protein level. It may increase degradation of GREM1. It may decrease the function of GREM1 by inhibitory modification. It may decrease the transcription of a molecule enhancing GREM1 function. It may disrupt DNA encoding GREM1 or a molecule enhancing GREM1 function, using an agent such as a zinc finger nuclease.

The antagonist may be an agent interacting with Gremlin-1. An agent that interacts with Gremlin-1 is typically an agent which binds Gremlin-1. Agents that interact with Gremlin-1 may modulate Gremlin-1. An inhibitory modulating agent may have an effect on any of the functions of Gremlin-1, but typically reduces binding of Gremlin-1 to BMP (BMP 2/4/7). The antagonist may be a BMP-7 mimetic molecule Gremlin-1 is a negative regulator of BMP, so reduced binding increases signalling through BMP. An activating modulating agent may increase binding of Gremlin-1 to BMP.

BMP binding and signalling may be detected by any method known in the art. For example, the Examples of the present application describe a SMAD phosphorylation assay. SMAD1, 5 and 8 are phosphorylated upon BMP signalling. An increase in SMAD phosphorylation may therefore be used to determine increased BMP signalling, which may reflect a reduction in binding to Gremlin-1. The Examples also describe an Id1 reporter gene assay, where the Id1 gene is a target gene of BMP signalling. An increase in

recovery of the signal in this assay may therefore also be used to determine if an agent inhibits Gremlin-1 binding to BMP.

The antagonist may act by binding the active site of GREM1 or act allosterically by binding at a different site. The antagonist may act by binding a regulator or ligand for GREM1, to thereby reduce activation of GREM1. The antagonist may be reversible or irreversible.

A GREM1 antagonist may be a small molecule inhibitor, a peptide, a protein, an antibody, a polynucleotide, an oligonucleotide, an antisense RNA, small interfering RNA (siRNA) or small hairpin RNA (shRNA).

An antagonist of GREM1 may be an oligonucleotide which specifically hybridises to an mRNA encoding GREM1 or an mRNA encoding a molecule which enhances GREM1 activity. An antagonist of GREM1 may be a polynucleotide encoding any molecule that decreases GREM1 function. For example, the GREM1 antagonist may be a polynucleotide encoding an anti-GREM1 antibody described herein.

An antagonist of GREM1 may be an antibody which specifically binds to any target molecule (typically a protein) so as to decrease GREM1 function directly or indirectly. The antagonist may be an antibody specifically binding GREM1. In this aspect, the antibody may decrease GREM1 function by allosteric inactivation or by blocking interaction between its target and a ligand required for activity.

Interaction of an antagonist agent with protein residues may be determined by any appropriate method known in the art, such as distances between the residue and agent as determined by x-ray crystallography (typically less than 6 Å, or less than 4 Å). As discussed in the Examples below, the region of Gremlin-1 which may be targeted by a therapeutic may include amino acids Asp92-Leu99, Arg116-His130, Ser137-Ser142, Cys176-Cys178. These are within 6 Å of those mutated on the surface of Gremlin-1.

Antibody antagonists

The term "antibody" as referred to herein includes whole antibodies and any antigen binding fragment (*i.e.*, "antigen-binding portion") or single chains thereof. An antibody refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen-binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or

V_H) and a heavy chain constant region. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR).

The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.,* effector cells) and the first component (Clq) of the classical complement system.

An antibody used according to the invention may be a monoclonal antibody or a polyclonal antibody, and will typically be a monoclonal antibody. An antibody used according to the invention may be a chimeric antibody, a CDR-grafted antibody, a nanobody, a human or humanised antibody or an antigen-binding portion of any thereof. For the production of both monoclonal and polyclonal antibodies, the experimental animal is typically a non-human mammal such as a goat, rabbit, rat or mouse but the antibody may also be raised in other species.

Polyclonal antibodies may be produced by routine methods such as immunisation of a suitable animal, with the antigen of interest. Blood may be subsequently removed from the animal and the IgG fraction purified.

Antibodies against Gremlin-1 may be obtained, where immunisation of an animal is necessary, by administering the polypeptides to an animal, e.g. a non-human animal, using well-known and routine protocols, see for example Handbook of Experimental Immunology, D. M. Weir (ed.), Vol 4, Blackwell Scientific Publishers, Oxford, England, 1986). Many warm-blooded animals, such as rabbits, mice, rats, sheep, cows, camels or pigs may be immunized. However, mice, rabbits, pigs and rats are generally most suitable.

Monoclonal antibodies may be prepared by any method known in the art such as the hybridoma technique (Kohler & Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.,* 1983, Immunology Today, 4:72) and the EBV-hybridoma technique (Cole *et al.,* Monoclonal Antibodies and Cancer Therapy, pp77-96, Alan R Liss, Inc., 1985).

Antibodies used according to the invention may also be generated using single lymphocyte antibody methods by cloning and expressing immunoglobulin variable region

cDNAs generated from single lymphocytes selected for the production of specific antibodies by for example the methods described by Babcook, J. *et al.*, 1996, Proc. Natl. Acad. Sci. USA 93(15): 7843-7848l; WO92/02551; WO2004/051268 and WO2004/106377.

The antibodies can also be generated using various phage display methods known in the art and include those disclosed by Brinkman *et al.* (in J. Immunol. Methods, 1995, 182: 41-50), Ames *et al.* (J. Immunol. Methods, 1995, 184:177-186), Kettleborough *et al.* (Eur. J. Immunol. 1994, 24:952-958), Persic *et al.* (Gene, 1997 187 9-18), Burton *et al.* (Advances in Immunology, 1994, 57:191-280) and WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and US 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108.

Fully human antibodies are those antibodies in which the variable regions and the constant regions (where present) of both the heavy and the light chains are all of human origin, or substantially identical to sequences of human origin, but not necessarily from the same antibody. Examples of fully human antibodies may include antibodies produced, for example by the phage display methods described above and antibodies produced by mice in which the murine immunoglobulin variable and optionally the constant region genes have been replaced by their human counterparts e.g. as described in general terms in EP 0546073, U5,545,806, US 5,569,825, US 5,625,126, US 5,633,425, US 5,661,016, US 5,770,429, EP 0438474 and EP 0463151.

Alternatively, an antibody used according to the invention may be produced by a method comprising immunising a non-human mammal with a Gremlin-1 immunogen; obtaining an antibody preparation from said mammal; deriving therefrom monoclonal antibodies that recognise Gremlin-1.

The antibody molecules used according the present invention may comprise a complete antibody molecule having full length heavy and light chains or a fragment or antigen-binding portion thereof. The term "antigen-binding portion" of an antibody refers to one or more fragments of an antibody that retain the ability to selectively bind to an antigen. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. The antibodies and fragments and antigen binding portions thereof may be, but are not limited to Fab, modified Fab, Fab',

modified Fab', F(ab')$_2$, Fv, single domain antibodies (e.g. VH or VL or VHH), scFv, bi, tri or tetra-valent antibodies, Bis-scFv, diabodies, triabodies, tetrabodies and epitope-binding fragments of any of the above (see for example Holliger and Hudson, 2005, Nature Biotech. 23(9):1126-1136; Adair and Lawson, 2005, Drug Design Reviews - Online 2(3), 209-217). The methods for creating and manufacturing these antibody fragments are well known in the art (see for example Verma et al., 1998, Journal of Immunological Methods, 216, 165-181). Other antibody fragments for use in the present invention include the Fab and Fab' fragments described in International patent applications WO 2005/003169, WO 2005/003170 and WO 2005/003171 and Fab-dAb fragments described in International patent application WO2009/040562. Multi-valent antibodies may comprise multiple specificities or may be monospecific (see for example WO 92/22853 and WO 05/113605). These antibody fragments may be obtained using conventional techniques known to those of skill in the art, and the fragments may be screened for utility in the same manner as intact antibodies.

The constant region domains of the antibody molecule, if present, may be selected having regard to the proposed function of the antibody molecule, and in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgD, IgE, IgG or IgM domains. In particular, human IgG constant region domains may be used, especially of the IgG1 and IgG3 isotypes when the antibody molecule is intended for therapeutic uses and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the antibody molecule is intended for therapeutic purposes and antibody effector functions are not required.

An antibody used according to the invention may be prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (*e.g.,* a mouse) that is transgenic or transchromosomal for the immunoglobulin genes of interest or a hybridoma prepared therefrom, (b) antibodies isolated from a host cell transformed to express the antibody of interest, *e.g.,* from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of immunoglobulin gene sequences to other DNA sequences.

An antibody used according to the invention may be a human antibody or a humanised antibody. The term "human antibody", as used herein, is intended to include

antibodies having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. The human antibodies described herein may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.,* mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

Such a human antibody may be a human monoclonal antibody. Such a human monoclonal antibody may be produced by a hybridoma that includes a B cell obtained from a transgenic nonhuman animal, *e.g.,* a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

Human antibodies may be prepared by *in vitro* immunisation of human lymphocytes followed by transformation of the lymphocytes with Epstein-Barr virus.

The term “human antibody derivatives” refers to any modified form of the human antibody, e.g., a conjugate of the antibody and another agent or antibody.

The term “humanized antibody” is intended to refer to CDR-grafted antibody molecules in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Additional framework region modifications may be made within the human framework sequences.

As used herein, the term ‘CDR-grafted antibody molecule’ refers to an antibody molecule wherein the heavy and/or light chain contains one or more CDRs (including, if desired, one or more modified CDRs) from a donor antibody (e.g. a murine or rat monoclonal antibody) grafted into a heavy and/or light chain variable region framework of an acceptor antibody (e.g. a human antibody). For a review, see Vaughan *et al*, Nature Biotechnology, 16, 535-539, 1998. In one embodiment rather than the entire CDR being transferred, only one or more of the specificity determining residues from any one of the CDRs described herein above are transferred to the human antibody framework (see for example, Kashmiri *et al.*, 2005, Methods, 36, 25-34). In one embodiment only the specificity determining residues from one or more of the CDRs described herein above are

transferred to the human antibody framework. In another embodiment only the specificity determining residues from each of the CDRs described herein above are transferred to the human antibody framework.

When the CDRs or specificity determining residues are grafted, any appropriate acceptor variable region framework sequence may be used having regard to the class/type of the donor antibody from which the CDRs are derived, including mouse, primate and human framework regions. Suitably, the CDR-grafted antibody according to the present invention has a variable domain comprising human acceptor framework regions as well as one or more of the CDRs or specificity determining residues described above. Thus, provided in one embodiment is a neutralising CDR-grafted antibody wherein the variable domain comprises human acceptor framework regions and non-human donor CDRs.

Examples of human frameworks which can be used in the present invention are KOL, NEWM, REI, EU, TUR, TEI, LAY and POM (Kabat *et al., supra*). For example, KOL and NEWM can be used for the heavy chain, REI can be used for the light chain and EU, LAY and POM can be used for both the heavy chain and the light chain. Alternatively, human germline sequences may be used; these are available for example at: http://www.vbase2.org/ (see Retter *et al*, Nucl. Acids Res. (2005) 33 (supplement 1), D671-D674).

In a CDR-grafted antibody described herein, the acceptor heavy and light chains do not necessarily need to be derived from the same antibody and may, if desired, comprise composite chains having framework regions derived from different chains.

Also, in a CDR-grafted antibody described herein, the framework regions need not have exactly the same sequence as those of the acceptor antibody. For instance, unusual residues may be changed to more frequently occurring residues for that acceptor chain class or type. Alternatively, selected residues in the acceptor framework regions may be changed so that they correspond to the residue found at the same position in the donor antibody (see Reichmann *et al.*, 1998, Nature, 332, 323-324). Such changes should be kept to the minimum necessary to recover the affinity of the donor antibody. A protocol for selecting residues in the acceptor framework regions which may need to be changed is set forth in WO 91/09967.

It will also be understood by one skilled in the art that antibodies may undergo a variety of posttranslational modifications. The type and extent of these modifications often

depends on the host cell line used to express the antibody as well as the culture conditions. Such modifications may include variations in glycosylation, methionine oxidation, diketopiperazine formation, aspartate isomerization and asparagine deamidation. A frequent modification is the loss of a carboxy-terminal basic residue (such as lysine or arginine) due to the action of carboxypeptidases (as described in Harris, RJ. *Journal of Chromatography* 705:129-134, 1995).

In one embodiment the antibody heavy chain comprises a CH1 domain and the antibody light chain comprises a CL domain, either kappa or lambda.

Biological molecules, such as antibodies or fragments, contain acidic and/or basic functional groups, thereby giving the molecule a net positive or negative charge. The amount of overall "observed" charge will depend on the absolute amino acid sequence of the entity, the local environment of the charged groups in the 3D structure and the environmental conditions of the molecule. The isoelectric point (pI) is the pH at which a particular molecule or surface carries no net electrical charge. In one embodiment the antibody or fragment according to the present disclosure has an isoelectric point (pI) of at least 7. In one embodiment the antibody or fragment has an isoelectric point of at least 8, such as 8.5, 8.6, 8.7, 8.8 or 9. In one embodiment the pI of the antibody is 8. Programs such as ** ExPASY http://www.expasy.ch/tools/pi_tool.html (see Walker, The Proteomics Protocols Handbook, Humana Press (2005), 571-607) may be used to predict the isoelectric point of the antibody or fragment.

In order to characterise preferred Gremlin-1 epitopes, the inventors have crystallised human Gremlin-1 alone, and in complex with an antibody termed Ab 7326 (Fab fragments). Crystallisation of Gremlin-1 has allowed putative residues in the BMP binding site to be determined. Furthermore, crystallisation with Ab 7326, which is an allosteric inhibitory antibody, has allowed residues in the antibody epitope to be determined. Antibodies binding this epitope have particular potential as therapeutic agents in the treatment of diseases associated with Gremlin-1.

The preferred Ab 7326 antibody described herein has been identified to bind the following residues of Gremlin-1: Ile110 (131), Lys126 (147), Lys127 (148), Phe128 (149), Thr129 (150), Thr130 (151), Arg148 (169), Lys153 (174) and Gln154 (175), where Lys126 (147), Lys127 (148), Phe128 (149), Thr129 (150), Thr130 (151), Arg148 (169), Lys153 (174) and Gln154 (175) are present on one Gremlin-1 monomer and Ile110 (131)

is present on the second Gremlin-1 monomer. Numbering not in brackets is based on the structural file and (which matches the numbering of mouse Gremlin-2 based on structural alignment). The numbers in brackets represent the residues based on the UniProt entry O60565 of SEQ ID NO: 1. As discussed in the Examples section, these epitope residues were identified using NCONT analysis at 4 Å from the Gremlin-1-Ab 7326 Fab complex.

Antibodies described herein may therefore bind to an epitope which comprises at least one residue selected from Ile131, Lys147, Lys148, Phe149, Thr150, Thr151, Arg169, Lys174 and Gln175 (with residue numbering based on SEQ ID NO: 1). Antibodies described herein may bind an epitope which comprises 2, 3, 4, 5, 6, 7, 8 or all 9 of these residues (preferably at least 5 residues).

Antibodies described herein may also recognise an epitope where Ile131 is present on a different Gremlin-1 monomer to the other residues.

Although these residues are provided for a particular sequence of human Gremlin-1, the skilled person could readibly extrapolate the positions of these residues to to other corresponding Gremlin sequences (e.g. mouse) using routine techniques. Antibodies binding to epitopes comprising the corresponding residues within these other Gremlin sequences are therefore also provided by the invention.

To screen for antibodies that bind to a particular epitope, a routine cross-blocking assay such as that described in Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harb., NY) can be performed. Other methods include alanine scanning mutants, peptide blots (Reineke (2004) Methods Mol Biol 248:443-63), or peptide cleavage analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer (2000) Protein Science 9: 487-496). Such methods are well known in the art.

Antibody epitopes may also be determined by x-ray crystallography analysis. Antibodies of the present disclosure may therefore be assessed through x-ray crystallogray analysis of the antibody bound to Gremlin-1. Epitopes may, in particular, be identified in this way by determining residues on Gremlin-1 within 4Å of an antibody paratope residue.

An antibody as described herein may thus bind to an epitope on Gremlin-1 comprising at least one residue selected from Trp93, Phe117, Tyr119, Phe125, Tyr126 and Phe138, wherein the residue numbering is according to SEQ ID NO: 1. Further described herein is an antibody which binds an epitope comprising all of Trp93, Phe117, Tyr119,

Phe125, Tyr126 and Phe138. Additionally described is an antibody which binds an epitope which comprises the following residues: Ile131, Lys147, Lys148, Phe149, Thr150, Thr151, Arg169, Lys174 and Gln175. Preferably, Lys147, Lys148, Phe149, Thr150, Thr151, Arg169, Lys174 and Gln175 are located on one monomer of Gremlin-1 and Ile131 is located on the other monomer of Gremlin-1 (Gremlin-1 dimers bind to BMP dimers).

An antibody may bind an above Gremlin-1 residue if the antibody paratope is within 4 Å of the Gremlin-1 residue as determined by x-ray crystallography.

Antibodies which bind to an epitope disclosed herein may comprise at least one, at least two or all three heavy chain CDR sequences of SEQ ID NOS: 4 to 6 (HCDR1/HCDR2/HCDR3 respectively). These are the HCDR1/HCDR2/HCDR3 sequences of the Ab 7326 antibody of the Examples as determined using Kabat methodology.

The Kabat and Chothia methods for determining CDR sequences are well known in the art (as well as other techniques). CDR sequences may be determined using any appropriate method and in the present invention, whilst Kabat is typically employed, other techniques could be used as well. In the present instance, SEQ ID NO: 3 presents the Ab 7326 HCDR1 sequence as determined using a combined Chothia & Kabat defintion.

Antibodies used according to the invention may comprise at least one, at least two or all three light chain CDR sequences of SEQ ID NOS: 7 to 9 (LCDR1/LCDR2/LCDR3 respectively). These are the LCDR1/LCDR2/LCDR3 sequences of Ab 7326 using Kabat methodology.

The antibody preferably comprises at least a HCDR3 sequence of SEQ ID NO: 6.

Typically, the antibody comprises at least one heavy chain CDR sequence selected from SEQ ID NOS: 4 to 6 and at least one light chain CDR sequence selected from SEQ ID NOS 7 to 9. The antibody may comprise at least two heavy chain CDR sequences selected from SEQ ID NOS: 4 to 6 and at least two light chain CDR sequences selected from SEQ ID NOS: 7 to 9. The antibody typically comprises all three heavy chain CDR sequences of SEQ ID NOS: 4 to 6 (HCDR1/HCDR2/HCDR3 respectively) and all three light chain CDR sequences SEQ ID NOS: 7 to 9 (LCDR1/LCDR2/LCDR3 respectively). The antibodies may be chimeric, human or humanised antibodies.

The antibody may comprise a heavy chain variable region (HCVR) sequence of SEQ ID NO: 10 or 12 (the HCVR of Ab 7326 variants 1 and 2). The antibody may comprise a

light chain variable region (LCVR) sequence of SEQ ID NO: 11 or 13 (the LCVR of Ab 7326 variants 1 and 2). The antibody preferably comprises the heavy chain variable region sequence of SEQ ID NO: 10 or 12 and the light chain variable region sequence of SEQ ID NO: 11 or 13 (especially HCVR/LVCR pairs of SEQ ID NOs: 10/11 or 12/13).

The antibody may comprise a heavy chain (H-chain) sequence of

SEQ ID NO: 14 mouse full length IgG1 heavy chain variant 1, or

SEQ ID NO: 28 mouse full length IgG1 heavy chain variant 2, or

SEQ ID NO: 30 human full length IgG1 heavy chain variant 1, or

SEQ ID NO: 16 human full length IgG1 heavy chain variant 2, or

SEQ ID NO: 22 human full length IgG4P heavy chain variant 1, or

SEQ ID NO: 34 human full-length IgG4P heavy chain variant 2, or

SEQ ID NO: 18 Fab heavy chain variant 1, or

SEQ ID NO: 32 Fab heavy chain variant 2.

The antibody may comprise a light chain (L-chain) sequence of

SEQ ID NO: 15 mouse full length IgG1 light chain variant 1, or

SEQ ID NO: 29 mouse full length IgG1 light chain variant 2, or

SEQ ID NO: 31 human full length IgG1 light chain variant 1, or

SEQ ID NO: 17 human full length IgG1 light chain variant 2, or

SEQ ID NO: 23 human full length IgG4P light chain variant 1, or

SEQ ID NO: 35 human full-length IgG4P light chain variant 2, or

SEQ ID NO: 19 Fab light chain variant 1, or

SEQ ID NO: 33 Fab light chain variant 2.

In one example, the antibody comprises a heavy chain / light chain sequence pair of

SEQ ID NOs: 14/15 mouse full length IgG1 variant 1, or

SEQ ID NOs: 28/29 mouse full length IgG1 variant 2, or

SEQ ID NOs: 30/31 human full length IgG1 variant 1, or

SEQ ID NOs: 16/17 human full length IgG1 variant 2, or

SEQ ID NOs: 22/23 human full length IgG4P variant 1, or

SEQ ID NOs: 34/35 human full-length IgG4P variant 2, or

SEQ ID NOs: 18/19 Fab light chain variant 1, or

SEQ ID NOs: 32/33 Fab light chain variant 2.

The variant forms of corresponding sequences may be interchanged. For example, the antibody may comprise a heavy chain / light chain sequence pair of

SEQ ID NOs: 14/29 mouse full length IgG1 heavy chain variant 1/light chain variant 2, or

SEQ ID NOs: 28/15 mouse full length IgG1 heavy chain variant 2/light chain variant 1, or

SEQ ID NOs: 30/17 human full length IgG1 heavy chain variant 1/light chain variant 2, or

SEQ ID NOs: 16/31 human full length IgG1 heavy chain variant 2/light chain variant 1, or

SEQ ID NOs: 22/35 human full length IgG4P heavy chain variant 1/light chain variant 2, or

SEQ ID NOs: 34/23 human full-length IgG4P heavy chain variant 2/light chain variant 1, or

SEQ ID NOs: 18/33 Fab heavy chain variant 1/light chain variant 2, or

SEQ ID NOs: 32/19 Fab heavy chain variant 2/light chain variant 1.

The antibodies may be chimeric, human or humanised antibodies.

The antibody may alternatively be or may comprise a variant of one of the specific sequences recited above. The following description of antibody variants is also applicable to selection of GREM1 polypeptide variants as described above.

For example, a variant may be a substitution, deletion or addition variant of any of the above amino acid sequences.

A variant antibody may comprise 1, 2, 3, 4, 5, up to 10, up to 20 or more (typically up to a maximum of 50) amino acid substitutions and/or deletions from the specific sequences discussed above. “Deletion” variants may comprise the deletion of individual amino acids, deletion of small groups of amino acids such as 2, 3, 4 or 5 amino acids, or deletion of larger amino acid regions, such as the deletion of specific amino acid domains or other features. "Substitution" variants typically involve the replacement of one or more amino acids with the same number of amino acids and making conservative amino acid substitutions. For example, an amino acid may be substituted with an alternative amino acid having similar properties, for example, another basic amino acid, another acidic amino acid, another neutral amino acid, another charged amino acid, another hydrophilic amino acid, another hydrophobic amino acid, another polar amino acid, another aromatic amino acid or another aliphatic amino acid. Some properties of the 20 main amino acids which can be used to select suitable substituents are as follows:

Ala	aliphatic, hydrophobic, neutral	Met	hydrophobic, neutral
Cys	polar, hydrophobic, neutral	Asn	polar, hydrophilic, neutral
Asp	polar, hydrophilic, charged (-)	Pro	hydrophobic, neutral
Glu	polar, hydrophilic, charged (-)	Gln	polar, hydrophilic, neutral
Phe	aromatic, hydrophobic, neutral	Arg	polar, hydrophilic, charged (+)
Gly	aliphatic, neutral	Ser	polar, hydrophilic, neutral
His	aromatic, polar, hydrophilic, charged (+)	Thr	polar, hydrophilic, neutral
Ile	aliphatic, hydrophobic, neutral	Val	aliphatic, hydrophobic, neutral
Lys	polar, hydrophilic, charged(+)	Trp	aromatic, hydrophobic, neutral
Leu	aliphatic, hydrophobic, neutral	Tyr	aromatic, polar, hydrophobic

"Derivatives" or "variants" generally include those in which instead of the naturally occurring amino acid the amino acid which appears in the sequence is a structural analog thereof. Amino acids used in the sequences may also be derivatized or modified, e.g. labelled, providing the function of the antibody is not significantly adversely affected.

Derivatives and variants as described above may be prepared during synthesis of the antibody or by post- production modification, or when the antibody is in recombinant form using the known techniques of site- directed mutagenesis, random mutagenesis, or enzymatic cleavage and/or ligation of nucleic acids.

Variant antibodies may have an amino acid sequence which has more than about 60%, or more than about 70%, e.g. 75 or 80%, typically more than about 85%, e.g. more than about 90 or 95% amino acid identity to the amino acid sequences disclosed herein (particularly the HCVR/LCVR sequences and the H- and L-chain sequences). Furthermore, the antibody may be a variant which has more than about 60%, or more than about 70%, e.g. 75 or 80%, typically more than about 85%, e.g. more than about 90 or 95% amino acid identity to the HCVR/LCVR sequences and the H- and L-chain sequences disclosed herein, whilst retaining the exact CDRs disclosed for these sequences. Variants may retain at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the HCVR/LCVR sequences and to the H- and L-chain sequences disclosed herein (in some circumstances whilst retaining the exact CDRs).

Variants typically retain about 60% - about 99% identity, about 80% - about 99% identity, about 90% - about 99% identity or about 95% - about 99% identity. This level of amino acid identity may be seen across the full length of the relevant SEQ ID NO sequence or over a part of the sequence, such as across about 20, 30, 50, 75, 100, 150, 200 or more amino acids, depending on the size of the full length polypeptide.

In connection with amino acid sequences, "sequence identity" refers to sequences which have the stated value when assessed using ClustalW (Thompson *et al.*, 1994, supra) with the following parameters:

Pairwise alignment parameters -Method: accurate, Matrix: PAM, Gap open penalty: 10.00, Gap extension penalty: 0.10;

Multiple alignment parameters -Matrix: PAM, Gap open penalty: 10.00, % identity for delay: 30, Penalize end gaps: on, Gap separation distance: 0, Negative matrix: no, Gap extension penalty: 0.20, Residue-specific gap penalties: on, Hydrophilic gap penalties: on, Hydrophilic residues: GPSNDQEKR. Sequence identity at a particular residue is intended to include identical residues which have simply been derivatized.

Antibodies having specific sequences and variants which maintain the function or activity of these chains are therefore provided.

Antibodies may compete for binding to Gremlin-1 with, or bind to the same epitope as, those defined above in terms of H-chain/L-chain, HCVR/LCVR or CDR sequences. In particular, an antibody may compete for binding to Gremlin-1 with, or bind to the same epitope as, an antibody which comprises a HCDR1/HCDR2/HCDR3/LCDR1/LCDR2/LCDR3 sequence combination of SEQ ID NOs: 4/5/6/7/8/9. An antibody may compete for binding to Gremlin-1 with, or bind to the same epitope as, an antibody which comprises a HCVR and LCVR sequence pair of SEQ ID NOs: 10/11 or 12/13 or full length chains of SEQ ID Nos: 14/15 or 16/17.

The term “epitope” is a region of an antigen that is bound by an antibody. Epitopes may be defined as structural or functional. Functional epitopes are generally a subset of the structural epitopes and have those residues that directly contribute to the affinity of the interaction. Epitopes may also be conformational, that is, composed of non-linear amino acids. In certain embodiments, epitopes may include determinants that are chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-

dimensional structural characteristics, and/or specific charge characteristics.

One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference antibody by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope as a reference antibody of the invention, the reference antibody is allowed to bind to a protein or peptide under saturating conditions. Next, the ability of a test antibody to bind to the protein or peptide is assessed. If the test antibody is able to bind to the protein or peptide following saturation binding with the reference antibody, it can be concluded that the test antibody binds to a different epitope than the reference antibody. On the other hand, if the test antibody is not able to bind to protein or peptide following saturation binding with the reference antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference antibody of the invention.

To determine if an antibody competes for binding with a reference antibody, the above-described binding methodology is performed in two orientations. In a first orientation, the reference antibody is allowed to bind to a protein/peptide under saturating conditions followed by assessment of binding of the test antibody to the protein/peptide molecule. In a second orientation, the test antibody is allowed to bind to the protein/peptide under saturating conditions followed by assessment of binding of the reference antibody to the protein/peptide. If, in both orientations, only the first (saturating) antibody is capable of binding to the protein/peptide, then it is concluded that the test antibody and the reference antibody compete for binding to the protein/peptide. As will be appreciated by the skilled person, an antibody that competes for binding with a reference antibody may not necessarily bind to the identical epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the antigen. That is, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50%, 75%, 90% or even 99% as measured in a competitive binding assay (see, e.g., Junghans et al., Cancer Res, 1990:50:1495-1502). Alternatively, two antibodies have the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if

some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

Additional routine experimentation (e.g., peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, surface plasmon resonance, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art.

Antibodies can be tested for binding to Gremlin-1 by, for example, standard ELISA or Western blotting. An ELISA assay can also be used to screen for hybridomas that show positive reactivity with the target protein. The binding selectivity of an antibody may also be determined by monitoring binding of the antibody to cells expressing the target protein, for example by flow cytometry. Thus, a screening method may comprise the step of identifying an antibody that is capable of binding Gremlin-1 by carrying out an ELISA or Western blot or by flow cytometry.

Antibodies may selectively (or specifically) recognise Gremlin-1. An antibody, or other compound, "selectively binds" or "selectively recognises" a protein when it binds with preferential or high affinity to the protein for which it is selective but does not substantially bind, or binds with low affinity, to other proteins. The selectivity of an antibody may be further studied by determining whether or not the antibody binds to other related proteins as discussed above or whether it discriminates between them. Antibodies used according to the invention typically recognise human Gremlin-1.

Antibodies may also have cross-reactivity for related proteins, or for human Gremlin-1 and for Gremlin-1 from other species.

By specific (or selective), it will be understood that the antibody binds to the protein of interest with no significant cross-reactivity to any other molecule. Cross-reactivity may be assessed by any suitable method described herein. Cross-reactivity of an antibody may be considered significant if the antibody binds to the other molecule at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 100% as strongly as it binds to the protein of interest. An antibody that is specific (or selective) may bind to another molecule at less than about 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25% or 20%

the strength that it binds to the protein of interest. The antibody may bind to the other molecule at less than about 20%, less than about 15%, less than about 10% or less than about 5%, less than about 2% or less than about 1% the strength that it binds to the protein of interest.

Anti-gremlin antibodies have been previously described, for example WO2014/159010A1 (Regeneron) describes anti-gremlin antibodies that inhibit Gremlin-1 activity, with binding affinity K_D values ranging from 625pM to 270nM at 25°C. Ciuclan et al (2013) describe an anti-Gremlin-1 monoclonal antibody with a binding affinity K_D 5.6 x 10^{-10} M.

The anti-Gremlin-1 antibodies newly described herein (and also in PCT/GB2017/083650 filed 19 December 2017, incorporated herein by reference in its entirety) are allosteric inhibitors of Gremlin-1 activity, and bind to a novel epitope as described above, distal from the BMP binding site. The antibodies bind to Gremlin-1 with exceptionally high affinity with Kd values <100pM. The antibodies therefore represent a significant improvement over currently available antibodies and are expected to be particularly useful for the treatment of Gremlin-1 mediated diseases.

Thus, antibodies suitable for use with the present invention may have a high affinity binding for (human) Gremlin-1. The antibody may have a dissociation constant (K_D) of less than <1 nM, and preferably <500 pM. In one example, the antibody has a dissociation constant (K_D) of less than 200pM. In one example, the antibody has a dissociation constant (K_D) of less than 100pM. A variety of methods can be used to determine the binding affinity of an antibody for its target antigen such as surface plasmon resonance assays, saturation assays, or immunoassays such as ELISA or RIA, as are well known to persons of skill in the art. An exemplary method for determining binding affinity is by surface plasmon resonance analysis on a BIAcore™ 2000 instrument (Biacore AB, Freiburg, Germany) using CM5 sensor chips, as described by Krinner et al., (2007) Mol. Immunol. February; 44 (5):916-25. (Epub 2006 May 11)).

Antibodies used according to the invention are typically inhibitory antibodies. Gremlin-1 negatively regulates BMP-2, 4 and 7, so inhibition of Gremlin-1 results in increased signalling through BMP.

As mentioned above, the Examples of the present application describe two functional assays for screening whether an antibody is capable of inhibiting Gremlin 1,

namely the SMAD phosphorylation assay and the Hek Id1 reporter gene assay. Typically, an inhibitory antibody restores SMAD phosphorylation and/or restores signalling of BMP in the Hek Id1 reporter gene assay. SMAD phosphorylation may be restored to at least 80 %, 90 % or 100 % when compared with a BMP control. In the Hek Id1 reporter gene assay, an inhibitory antibody may have an IC_{50} of less than 10 nM, preferably less than 5 nM.

Once a suitable antibody has been identified and selected, the amino acid sequence of the antibody may be identified by methods known in the art. The genes encoding the antibody can be cloned using degenerate primers. The antibody may be recombinantly produced by routine methods.

The present disclosure also provides an isolated DNA sequence encoding the heavy and/or light chain variable regions(s) (or the full length H- and L-chains) of an antibody molecule newly described herein.

A variant polynucleotide may comprise 1, 2, 3, 4, 5, up to 10, up to 20, up to 30, up to 40, up to 50, up to 75 or more nucleic acid substitutions and/or deletions from any of the nucleic acid sequences (including GREM1 and anti-GREM1 antibody nucleic acid sequences) given in the sequence listing. Generally, a variant has 1-20, 1-50, 1-75 or 1-100 substitutions and/or deletions.

Suitable variants may be at least about 70% homologous to a polynucleotide of any one of nucleic acid sequences disclosed herein, typically at least about 80 or 90% and more suitably at least about 95%, 97% or 99% homologous thereto. Variants may retain at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity. Variants typically retain about 60% - about 99% identity, about 80% - about 99% identity, about 90% - about 99% identity or about 95% - about 99% identity. Homology and identity at these levels is generally present at least with respect to the coding regions of the polynucleotides. Methods of measuring homology are well known in the art and it will be understood by those of skill in the art that in the present context, homology is calculated on the basis of nucleic acid identity. Such homology may exist over a region of at least about 15, at least about 30, for instance at least about 40, 60, 100, 200 or more contiguous nucleotides (depending on the length). Such homology may exist over the entire length of the unmodified polynucleotide sequence.

Methods of measuring polynucleotide homology or identity are known in the art. For example the UWGCG Package provides the BESTFIT program which can be used to calculate homology (e.g. used on its default settings) (Devereux *et al* (1984) Nucleic Acids Research 12, p387-395).

The PILEUP and BLAST algorithms can also be used to calculate homology or line up sequences (typically on their default settings), for example as described in Altschul S.F. (1993) J Mol Evol 36:290-300; Altschul, S, F *et al* (1990) J Mol Biol 215:403-10.

Software for performing BLAST analysis is publicly available through the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul *et al*, supra). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extensions for the word hits in each direction are halted when: the cumulative alignment score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) Proc. Natl. Acad. Sci. USA 89:10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g., Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5787. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, typically less than about 0.1, suitablyless than about 0.01, and most suitably less than about 0.001. For

example, the smallest sum probability may be in the range of about 1 - about 0.001, often about 0.01 - about 0.001.

The homologue may differ from a sequence in the relevant polynucleotide by less than about 3, 5, 10, 15, 20 or more mutations (each of which may be a substitution, deletion or insertion). For example, the homologue may differ by 3-50 mutations, often 3-20 mutations. These mutations may be measured over a region of at least 30, for instance at least about 40, 60 or 100 or more contiguous nucleotides of the homologue.

In one embodiment, a variant sequence may vary from the specific sequences given in the sequence listing by virtue of the redundancy in the genetic code. The DNA code has 4 primary nucleic acid residues (A, T, C and G) and uses these to "spell" three letter codons which represent the amino acids the proteins encoded in an organism's genes. The linear sequence of codons along the DNA molecule is translated into the linear sequence of amino acids in the protein(s) encoded by those genes. The code is highly degenerate, with 61 codons coding for the 20 natural amino acids and 3 codons representing "stop" signals. Thus, most amino acids are coded for by more than one codon - in fact several are coded for by four or more different codons. A variant polynucleotide of the invention may therefore encode the same polypeptide sequence as another polynucleotide of the invention, but may have a different nucleic acid sequence due to the use of different codons to encode the same amino acids.

The DNA sequence may comprise synthetic DNA, for instance produced by chemical processing, cDNA, genomic DNA or any combination thereof.

DNA sequences which encode an antibody molecule described herein can be obtained by methods well known to those skilled in the art. For example, DNA sequences coding for part or all of the antibody heavy and light chains may be synthesised as desired from the determined DNA sequences or on the basis of the corresponding amino acid sequences.

General methods by which the vectors may be constructed, transfection methods and culture methods are well known to those skilled in the art. In this respect, reference is made to "Current Protocols in Molecular Biology", 1999, F. M. Ausubel (ed), Wiley Interscience, New York and the Maniatis Manual produced by Cold Spring Harbor Publishing.

Nucleic acid antagonists

A polynucleotide, such as a nucleic acid, is a polymer comprising two or more nucleotides. The nucleotides can be naturally occurring or artificial. A nucleotide typically contains a nucleobase, a sugar and at least one linking group, such as a phosphate, 2'O-methyl, 2' methoxy-ethyl, phosphoramidate, methylphosphonate or phosphorothioate group. The nucleobase is typically heterocyclic. Nucleobases include, but are not limited to, purines and pyrimidines and more specifically adenine (A), guanine (G), thymine (T), uracil (U) and cytosine (C). The sugar is typically a pentose sugar. Nucleotide sugars include, but are not limited to, ribose and deoxyribose. The nucleotide is typically a ribonucleotide or deoxyribonucleotide. The nucleotide typically contains a monophosphate, diphosphate or triphosphate. Phosphates may be attached on the 5' or 3' side of a nucleotide.

Nucleotides include, but are not limited to, adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP), guanosine monophosphate (GMP), guanosine diphosphate (GDP), guanosine triphosphate (GTP), thymidine monophosphate (TMP), thymidine diphosphate (TDP), thymidine triphosphate (TTP), uridine monophosphate (UMP), uridine diphosphate (UDP), uridine triphosphate (UTP), cytidine monophosphate (CMP), cytidine diphosphate (CDP), cytidine triphosphate (CTP), 5-methylcytidine monophosphate, 5-methylcytidine diphosphate, 5-methylcytidine triphosphate, 5-hydroxymethylcytidine monophosphate, 5-hydroxymethylcytidine diphosphate, 5-hydroxymethylcytidine triphosphate, cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), deoxyadenosine monophosphate (dAMP), deoxyadenosine diphosphate (dADP), deoxyadenosine triphosphate (dATP), deoxyguanosine monophosphate (dGMP), deoxyguanosine diphosphate (dGDP), deoxyguanosine triphosphate (dGTP), deoxythymidine monophosphate (dTMP), deoxythymidine diphosphate (dTDP), deoxythymidine triphosphate (dTTP), deoxyuridine monophosphate (dUMP), deoxyuridine diphosphate (dUDP), deoxyuridine triphosphate (dUTP), deoxycytidine monophosphate (dCMP), deoxycytidine diphosphate (dCDP) and deoxycytidine triphosphate (dCTP), 5-methyl-2'-deoxycytidine monophosphate, 5-methyl-2'-deoxycytidine diphosphate, 5-methyl-2'-deoxycytidine triphosphate, 5-hydroxymethyl-2'-deoxycytidine monophosphate, 5-hydroxymethyl-2'-deoxycytidine diphosphate and 5-

hydroxymethyl-2'-deoxycytidine triphosphate. The nucleotides are preferably selected from AMP, TMP, GMP, UMP, dAMP, dTMP, dGMP or dCMP.

The nucleotides may contain additional modifications. In particular, suitable modified nucleotides include, but are not limited to, 2'amino pyrimidines (such as 2'-amino cytidine and 2'-amino uridine), 2'-hyrdroxyl purines (such as , 2'-fluoro pyrimidines (such as 2'-fluorocytidine and 2'fluoro uridine), hydroxyl pyrimidines (such as 5'-α-P-borano uridine), 2'-O-methyl nucleotides (such as 2'-O-methyl adenosine, 2'-O-methyl guanosine, 2'-O-methyl cytidine and 2'-O-methyl uridine), 4'-thio pyrimidines (such as 4'-thio uridine and 4'-thio cytidine) and nucleotides have modifications of the nucleobase (such as 5-pentynyl-2'-deoxy uridine, 5-(3-aminopropyl)-uridine and 1,6-diaminohexyl-N-5-carbamoylmethyl uridine).

The nucleotides in the polynucleotide may be attached to each other in any manner. The nucleotides may be linked by phosphate, 2'O-methyl, 2' methoxy-ethyl, phosphoramidate, methylphosphonate or phosphorothioate linkages. The nucleotides are typically attached by their sugar and phosphate groups as in nucleic acids. The nucleotides may be connected via their nucleobases as in pyrimidine dimers.

The GREM1 antagonist may be a polynucleotide encoding an anti-GREM1 antibody described herein.

The polynucleotide can be a nucleic acid, such as deoxyribonucleic acid (DNA) or a ribonucleic acid (RNA). The polynucleotide may be any synthetic nucleic acid known in the art, such as peptide nucleic acid (PNA), glycerol nucleic acid (GNA), threose nucleic acid (TNA), locked nucleic acid (LNA), morpholino nucleic acid or other synthetic polymers with nucleotide side chains. The polynucleotide may be single stranded or double stranded.

The polynucleotide sequence may be cloned into any suitable expression vector. In an expression vector, the polynucleotide sequence encoding a construct is typically operably linked to a control sequence which is capable of providing for the expression of the coding sequence by the host cell. Such expression vectors can be used to express a construct.

In one embodiment, the anti-GREM1 antagonist is a polynucleotide encoding an anti-GREM1 antibody described herein. The polynucleotide may be provided for use in

gene therapy. The polynucleotide may be provided in any suitable vector capable of providing for expression of the anti-GREM1 antibody *in vivo*.

The polynucleotide encoding the anti-GREM1 antibody may be a DNA sequence. The DNA sequence may be provided in any suitable vector, *e.g.* an expression vector, for administration to a subject in need thereof. For example, the DNA sequence may be administered to the subject in an expression vector capable of providing for expression of the anti-GREM1 antibody *in vivo*. The expression vector may be a viral expression vector, such as an adeno-associated virus (AAV) vector. In one embodiment, the anti-GREM1 antagonist is a DNA sequence which encodes an anti-GREM1 antibody described herein. In one embodiment, the anti-GREM1 antagonist is a DNA sequence for use in gene therapy, wherein the DNA sequence encodes an anti-GREM1 antibody described herein. In one embodiment, the anti-GREM1 antagonist is an AAV comprising a DNA sequence which encodes an anti-GREM1 antibody described herein. In one embodiment, the anti-GREM1 antagonist is an AAV for use in gene therapy, wherein the AAV comprises a DNA sequence which encodes an anti-GREM1 antibody described herein.

The polynucleotide encoding the anti-GREM1 antibody may be an RNA sequence. The RNA sequence may be administered to a subject in need thereof in any suitable vector. The RNA sequence may be a messenger RNA (mRNA) sequence. The mRNA sequence may be administered to a subject in need thereof in a stabilised form. For example the mRNA sequence may be provided in a lipid nanoparticle (LNP) composition. The LNP composition may comprise any suitable LNPs capable of encapsulating the mRNA sequence to provide for increased stability of said mRNA sequence. Thus, in one embodiment, the anti-GREM1 antagonist is a stabilised mRNA sequence encoding an anti-GREM1 antibody described herein. In one embodiment, the anti-GREM1 antagonist is a stabilised mRNA sequence for use in gene therapy, wherein the mRNA sequence encodes an anti-GREM1 antibody described herein. In one embodiment, the anti-GREM1 antagonist is a LNP composition which comprises an mRNA encoding an anti-GREM1 antibody described herein. In one embodiment, the anti-GREM1 antagonist is an LNP composition for use in gene therapy, wherein the LNP composition comprises an mRNA encoding an anti-GREM1 antibody described herein.

The term "*operably linked*" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A

control sequence "*operably linked*" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. Multiple copies of the same or different polynucleotide may be introduced into the vector.

The expression vector may then be introduced into a suitable host cell. Thus, a construct can be produced by inserting a polynucleotide sequence encoding a construct into an expression vector, introducing the vector into a compatible bacterial host cell, and growing the host cell under conditions which bring about expression of the polynucleotide sequence.

A GREM1 antagonist which is nucleic acid-based may reduce expression of GREM1. Antisense and RNA interference (RNAi) technology for knocking down protein expression are well known in the art and standard methods can be employed to knock down expression of a molecule of interest. Both antisense and siRNA technology interfere with mRNA. Antisense oligonucleotides interfere with mRNA by binding to (hybridising with) a section of the mRNA. The antisense oligonucleotide is therefore designed to be complementary to the mRNA (although the oligonucleotide does not have to be 100% complementary as discussed below). In other words, the antisense oligonucleotide may be a section of the cDNA. Again, the oligonucleotide sequence may not be 100% identical to the cDNA sequence. This is also discussed below. RNAi involves the use of double-stranded RNA, such small interfering RNA (siRNA) or small hairpin RNA (shRNA), which can bind to the mRNA and inhibit protein expression.

Accordingly, the antagonist may be a oligonucleotide which specifically hybridises to an mRNA encoding GREM1, such as the encoding sequence of SEQ ID NO: 36 or SEQ ID NO: 37 or a variant thereof. An oligonucleotide "*specifically hybridises*" to a target sequence when it hybridises with preferential or high affinity to the target sequence but does not substantially hybridise, does not hybridise or hybridises with only low affinity to other sequences. More preferably, the oligonucleotide hybridises to the target sequence with a T_m that is at least 5 °C, at least at least 10 °C, at least 20 °C, at least 30 °C or at least 40 °C, greater than its T_m for other nucleic acids. Conditions that permit the hybridisation are well-known in the art (for example, Sambrook et al., 2001, Molecular Cloning: a laboratory manual, 3rd edition, Cold Spring Harbour Laboratory Press; and Current Protocols in Molecular Biology, Chapter 2, Ausubel et al., Eds., Greene Publishing and

Wiley-Interscience, New York (1995)). The hybridisation conditions may be stringent conditions as described in the art.

Oligonucleotides are short nucleotide polymers which typically have 50 or fewer nucleotides, such 40 or fewer, 30 or fewer, 22 or fewer, 21 or fewer, 20 or fewer, 10 or fewer or 5 or fewer nucleotides. The oligonucleotide used may be 20 to 25 nucleotides in length, more preferably 21 or 22 nucleotides in length. The nucleotides can be naturally occurring or artificial. The nucleotides can be any of those described above.

The GREM1 antagonist may be an antibody that binds to GREM1, typically specifically binding GREM1. An antibody "specifically binds" to a protein when it binds with preferential or high affinity to that protein but does not substantially bind, does not bind or binds with only low affinity to other proteins. For instance, an antibody "specifically binds" a target molecule when it binds with preferential or high affinity to that target but does not substantially bind, does not bind or binds with only low affinity to other human proteins.

An antibody binds with preferential or high affinity if it binds with a Kd of 1 x 10-7 M or less, more preferably 5 x 10-8 M or less, more preferably 1 x 10-8 M or less or more preferably 5 x 10-9 M or less. An antibody binds with low affinity if it binds with a Kd of 1 x 10-6 M or more, more preferably 1 x 10-5 M or more, more preferably 1 x 10-4 M or more, more preferably 1 x 10-3 M or more, even more preferably 1 x 10-2 M or more.

The antibody may be, for example, a monoclonal antibody, a polyclonal antibody, a single chain antibody, a chimeric antibody, a bispecific antibody, a CDR-grafted antibody or a humanized antibody. The antibody may be an intact immunoglobulin molecule or a fragment thereof such as a Fab, $F(ab')_2$ or Fv fragment.

Patient

Any patient may be treated in accordance with the invention. The patient is typically human. However, the patient may be another mammalian animal, such as a commercially farmed animal, such as a horse, a cow, a sheep, a fish, a chicken or a pig, a laboratory animal, such as a mouse or a rat, or a pet, such as a guinea pig, a hamster, a rabbit, a cat or a dog.

Pharmaceutical Compositions, Dosages and Dosage Regimes

A GREM1 antagonist of the invention may be provided in a pharmaceutical composition. The pharmaceutical composition will normally be sterile and will typically include a pharmaceutically acceptable carrier and/or adjuvant. A pharmaceutical composition of the present invention may additionally comprise a pharmaceutically acceptable adjuvant and/or carrier.

As used herein, "*pharmaceutically acceptable carrier*" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The carrier may be suitable for parenteral, e.g. intravenous, intramuscular, intradermal, intraocular, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. Alternatively, the carrier may be suitable for non-parenteral administration, such as a topical, epidermal or mucosal route of administration. The carrier may be suitable for oral administration. Depending on the route of administration, the modulator may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

The pharmaceutical compositions of the invention may include one or more pharmaceutically acceptable salts. A "*pharmaceutically acceptable salt*" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects. Examples of such salts include acid addition salts and base addition salts.

Pharmaceutically acceptable carriers comprise aqueous carriers or diluents. Examples of suitable aqueous carriers that may be employed in the pharmaceutical compositions of the invention include water, buffered water and saline. Examples of other carriers include ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. In many cases, it will be desirable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration.

Pharmaceutical compositions of the invention may comprise additional active ingredients.

Also within the scope of the present disclosure are kits comprising antagonists described herein and instructions for use. The kit may further contain one or more additional reagents, such as an additional therapeutic or prophylactic agent as discussed herein.

The antagonists described herein or formulations or compositions thereof may be administered for prophylactic and/or therapeutic treatments.

In therapeutic applications, compounds are administered to a subject already suffering from a disorder or condition as described above, in an amount sufficient to cure, alleviate or partially arrest the condition or one or more of its symptoms. Such therapeutic treatment may result in a decrease in severity of disease symptoms, or an increase in frequency or duration of symptom-free periods. An amount adequate to accomplish this is defined as a "*therapeutically effective amount*".

In prophylactic applications, formulations are administered to a subject at risk of a disorder or condition as described above, in an amount sufficient to prevent or reduce the subsequent effects of the condition or one or more of its symptoms. An amount adequate to accomplish this is defined as a "*prophylactically effective amount*". Effective amounts for each purpose will depend on the severity of the disease or injury as well as the weight and general state of the subject.

A subject for administration may be a human or non-human animal. The term "*non-human animal*" includes all vertebrates, *e.g.,* mammals and non-mammals, such as non-human primates, sheep, dogs, cats, horses, cows, chickens, amphibians, reptiles, etc. Administration to humans is typical.

An antagonist or pharmaceutical composition of the invention may be administered via one or more routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Examples of routes of administration for compounds or pharmaceutical compositions of the invention include intravenous, intramuscular, intradermal, intraocular, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "*parenteral administration*" as used herein means modes of administration other

than enteral and topical administration, usually by injection. Alternatively, antibody/modulatory agent or pharmaceutical composition of the invention can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration. The antibody/modulatory agent or pharmaceutical composition of the invention may be for oral administration.

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

A suitable dose may be, for example, in the range of from about 0.01µg/kg to about 1000mg/kg body weight, typically from about 0.1µg/kg to about 100mg/kg body weight, of the patient to be treated. For example, a suitable dosage may be from about 1µg/kg to about 10mg/kg body weight per day or from about 10 µg/kg to about 5 mg/kg body weight per day.

Dosage regimens may be adjusted to provide the optimum desired response (*e.g.,* a therapeutic response). For example, a single dose may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

Administration may be in single or multiple doses. Multiple doses may be administered via the same or different routes and to the same or different locations. Alternatively, doses can be via a sustained release formulation, in which case less frequent administration is required. Dosage and frequency may vary depending on the half-life of the antagonist in the patient and the duration of treatment desired.

As mentioned above, modulators/antibodies or pharmaceutical compositions of the invention may be co-administered with one or other more other therapeutic agents.

Combined administration of two or more agents may be achieved in a number of different ways. Both may be administered together in a single composition, or they may be administered in separate compositions as part of a combined therapy. For example, the one may be administered before or separately, after or sequential, or concurrently or simultaneously with the other.

Pharmaceutical Compositions and Modes of Administration

The antagonists for use in the methods of treatment described herein may be formulated in pharmaceutical compositions. These compositions may comprise, in addition to the therapeutically active ingredient(s), a pharmaceutically acceptable excipient, carrier, diluent, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The pharmaceutical carrier or diluent may be, for example, an isotonic solution.

The precise nature of the carrier or other material may depend on the route of administration, e.g. oral, intravenous, cutaneous or subcutaneous, nasal, intramuscular and intraperitoneal routes. For example, solid oral forms may contain, together with the active substance, diluents, e.g. lactose, dextrose, saccharose, cellulose, corn starch or potato starch; lubricants, e.g. silica, talc, stearic acid, magnesium or calcium stearate, and/or polyethylene glycols; binding agents; e.g. starches, gum arabic, gelatin, methylcellulose, carboxymethylcellulose or polyvinyl pyrrolidone; disaggregating agents, e.g. starch, alginic acid, alginates or sodium starch glycolate; effervescing mixtures; dyestuffs; sweeteners; wetting agents, such as lecithin, polysorbates, laurylsulphates; and, in general, non-toxic and pharmacologically inactive substances used in pharmaceutical formulations. Such pharmaceutical preparations may be manufactured in known manner, for example, by

means of mixing, granulating, tabletting, sugar-coating, or film-coating processes.

Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10% to 95% of active ingredient, preferably 25% to 70%. Where the pharmaceutical composition is lyophilised, the lyophilised material may be reconstituted prior to administration, e.g. a suspension. Reconstitution is preferably effected in buffer.

Capsules, tablets and pills for oral administration to an individual may be provided with an enteric coating comprising, for example, Eudragit "S", Eudragit "L", cellulose acetate, cellulose acetate phthalate or hydroxypropylmethyl cellulose.

Liquid dispersions for oral administration may be syrups, emulsions or suspensions. The syrups may contain as carriers, for example, saccharose or saccharose with glycerine and/or mannitol and/or sorbitol.

Suspensions and emulsions may contain as carrier, for example a natural gum, agar, sodium alginate, pectin, methylcellulose, carboxymethylcellulose, or polyvinyl alcohol. The suspensions or solutions for intramuscular injections may contain, together with the active substance, a pharmaceutically acceptable carrier, e.g. sterile water, olive oil, ethyl oleate, glycols, e.g. propylene glycol, and if desired, a suitable amount of lidocaine hydrochloride.

Solutions for intravenous administration or infusion may contain as carrier, for example, sterile water or preferably they may be in the form of sterile, aqueous, isotonic saline solutions.

For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1% to 2%.

Polynucleotide or oligonucleotide inhibitors maybe naked nucleotide sequences or be in combination with cationic lipids, polymers or targeting systems. They may be delivered by any available technique. For example, the polynucleotide or oligonucleotide may be introduced by needle injection, preferably intradermally, subcutaneously or intramuscularly. Alternatively, the polynucleotide or oligonucleotide may be delivered directly across the skin using a delivery device such as particle-mediated gene delivery.

The polynucleotide or oligonucleotide may be administered topically to the skin, or to mucosal surfaces for example by intranasal, oral, or intrarectal administration.

Uptake of polynucleotide or oligonucleotide constructs may be enhanced by several known transfection techniques, for example those including the use of transfection agents. Examples of these agents include cationic agents, for example, calcium phosphate and DEAE-Dextran and lipofectants, for example, lipofectam and transfectam. The dosage of the polynucleotide or oligonucleotide to be administered can be altered.

Administration is typically in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy), this being sufficient to show benefit to the individual, e.g. an effective amount to prevent or delay onset of the disease or condition, to ameliorate one or more symptoms, to induce or prolong remission, or to delay relapse or recurrence.

The dose may be determined according to various parameters, especially according to the substance used; the age, weight and condition of the individual to be treated; the route of administration; and the required regimen. A physician will be able to determine the required route of administration and dosage for any particular individual. A typical daily dose is from about 0.1 to 50 mg per kg of body weight dependent on the conditions mentioned above. The dose may be provided as a single dose or may be provided as multiple doses, for example taken at regular intervals, for example 2, 3 or 4 doses administered hourly. Typically polynucleotide or oligonucleotide inhibitors are administered in the range of 1 pg to 1 mg, preferably to 1 pg to 10 μg nucleic acid for particle mediated delivery and 10 μg to 1 mg for other routes.

Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 20th Edition, 2000, pub. Lippincott, Williams & Wilkins.

Therapeutic combinations

A composition of the invention as described above may be used/administered alone or in combination with other therapeutic compositions or treatments, for example as adjunct therapy. The other therapeutic compositions or treatments may for example be one or more of those discussed herein, and may be administered either simultaneously or sequentially with the composition of the invention.

As discussed above, GREM1 antagonists have particular utility in combination treatments, since they may be used to sensitise a cancer or tumour to another anti-cancer agent, such as a chemotherapeutic agent, or to another cancer therapy, such as radiotherapy or surgery. The cancer may be resistant to the other anti-cancer agent or cancer therapy in the absence of the GREM1 antagonist.

Thus, an anti-GREM1 antagonist may be used in combination with any other cancer therapy or any other therapeutic agent for a cancer, such as a chemotherapeutic agent . The other cancer therapy may be selected from any known therapy for the relevant cancer, such as any known therapy for colorectal cancer. The other cancer therapy may be a radiotherapy. Suitable radiotherapy treatments are described for example in Van Cutsem (and others) Annals of Oncology, 2014. Vol 25, Issue 3. The radiotherapy may be carried out before surgery on a cancer or after surgery on a cancer. The radiotherapy may be adjuvant radiotherapy. The radiotherapy may be carried out in combination with a chemotherapy, for example administration of a chemotherapeutic agent as described below.

The other therapeutic agent for a cancer, such as a chemotherapeutic agent may be selected from any known therapeutic agent for the relevant cancer, including any known chemotherapeutic agent or combination of chemotherapeutic agents for the relevant cancer. For example, a GREM1 antagonist may be used in combination with one or more of 5-fluoruracil, oxaliplatin, irinotecan, and folinic acid, particularly in treatments of colorectal cancer. Further examples of combination treatments combining GREM1 antagonists with other anti-cancer agents for treatment of colorectal cancer and multiple myeloma are described below in the context of compositions and kits for treatment. The cancer may be resistant to one or more chemotherapeutic agents (such as one of the above chemotherapeutic agents) when not administered in combination with a GREM1 antagonist. A GREM1 antagonist may be used in combination with cetuximab, nivolumab or bevacizumab. A bispecific antibody combining an anti-GREM1 specificity and a nivolumab or bevacizumab specificity may be administered.

As part of the above aspects, the invention provides an anti-GREM1 antagonist for use in a method of treatment and/or prevention of cancer according to the invention, wherein the method comprises separate, sequential or simultaneous administration of a chemotherapeutic agent. The invention also provides an anti-GREM1 antagonist for use in

a method of treatment and/or prevention of cancer according to the invention, wherein the method comprises separate, sequential or simultaneous radiotherapy. The invention further provides a chemotherapeutic agent for use in a method for the treatment of a cancer having stromal GREM1 overexpression wherein the method comprises separate, sequential or simultaneous administration of an anti-GREM1 antagonist. The invention also provides a chemotherapeutic agent for use in a method for the prevention or treatment of a cancer having epithelial GREM1 overexpression wherein the method comprises separate, sequential or simultaneous administration of an anti-GREM1 antagonist.

Additionally provided is a composition or kit comprising an anti-GREM1 antagonist and an additional anti-cancer agent. The additional anti-cancer agent may be a targeted therapeutic or a chemotherapeutic agent. The additional anti-cancer agent may be any anti-cancer agent described above. More than one such additional anti-cancer agents may be incorporated in the composition or kit. The composition or kit may comprise an anti-GREM1 antagonist and one or more anti-cancer agents selected from: 5-fluoruracil, oxaliplatin, irinotecan, and folinic acid, cetuximab, nivolumab and bevacizumab, particularly as part of a composition or kit for treatment of colorectal cancer. A composition or kit for treatment of multiple myeloma may comprise an anti-GREM1 antagonist and one or more of an anti-CD38 antibody (such as daratumumab), an anti-SLAMF7 antibody (such as elotuzumab) and/or an anti-IL-6 antibody (such as siltuximab). A bispecific antibody combining an anti-GREM1 specificity and one of the other above specificities may be provided in a composition or kit. A preferred combination comprises an anti-GREM1 antagonist and an anti IL-6 antibody (preferably siltuximab). A composition or kit for treatment of multiple myeloma may comprise an anti-GREM1 antagonist and bortezumib and/or iMID (lenalidomide/pomalenomide) or an analogue of either thereof. The anti-GREM1 antagonist in any of the above compositions and kits may preferably be an anti-GREM1 antibody.

Therapeutic indications

Antagonists of the present invention are used in treating or preventing cancer.

Prevention of cancer may include preventing the subject from ever being diagnosed with cancer or deferring the onset of cancer. Prevention of cancer may also include prevention of relapse or recurrence of cancer in a subject who has been previously

diagnosed with cancer. Prevention of cancer may additionally include increasing the survival of a subject who has not been diagnosed with cancer or who has been previously diagnosed with cancer.

Treatment of cancer may ameliorate one or more symptoms of, induce or prolong remission from, or delay relapse or recurrence of the cancer. Treatment may cure, alleviate or partially arrest the cancer. It may result in a decrease in severity of disease symptoms, or an increase in frequency or duration of symptom-free periods. Treatment of cancer may also include preventing a cancer (*e.g.* an established cancer) from spreading from its initial site within a patient's body to one or more secondary sites within the patient's body. Thus, treatment of cancer may include prevention of the dissemination or the metastasis of an existing cancer. Treatment of colorectal cancer may result in a reduction in polyp burden (number of polyps) as assayed for example by endoscopy. Treatment of multiple myeloma may result in a reduction in bone marrow tumour burden, for example as assayed by MRI/CT scan, and/or a reduction in plasma cell burden, which may be determined following bone marrow biopsy, and/or reduction of one or more serum markers such as monoclonal antibody production (paraprotein) and serum free light chain (FLC) ratio, and/or reduction in associated lytic lesions of the skeleton, which may be detectable by radiography.

Detection and diagnosis

Based on the correlation between stromal GREM1 and cancer, the present invention also provides for additional means of detection and diagnosis of cancer, and for prognosis of cancer and prediction of responsiveness of a cancer to a treatment.

The invention thus provides a method for detecting cancer in a patient, the method comprising measuring stromal expression of GREM1 in the patient, wherein stromal overexpression of GREM1 indicates that the patient comprises a cancer. The invention also provides a method for prognosing a cancer in a patient, the method comprising determining whether or not the cancer comprises stromal overexpression of GREM1, wherein stromal overexpression of GREM1 in the cancer indicates that the patient has a worse prognosis than in the situation of normal stromal expression of GREM1. The cancer may be any cancer described herein. The cancer is preferably colorectal cancer, typically comprising a desmoplastic stroma, multiple myeloma or breast cancer.

Diagnosis includes determining whether or not an individual has a cancer or tumour and/or determining the severity of the cancer or tumour.

Prognosis includes predicting whether or not an individual will develop a cancer or tumour, whether or not they will need treatment, the type of treatment the individual will need, whether or not they will respond to a treatment, whether or not and/or when they will suffer a cancer episode, recurrence or relapse, and the severity or duration of a symptom or a cancer episode, recurrence or relapse. The method of prognosis may predict whether or not an individual in remission from cancer will have a recurrence. Predicting whether or not the individual will have a recurrence includes determining the likelihood that the individual will have a recurrence, and/or predicting when they will have a recurrence. The invention further provides a method for determining whether or not a patient having or suspected of having or being at risk of developing cancer is likely to respond to treatment with a chemotherapeutic agent, which method comprises measuring stromal expression of GREM1 in the patient, and thereby predicting whether or not the patient is likely to respond to treatment with the chemotherapeutic agent.

The invention additionally provides a method for determining whether or not a patient having or suspected of having or being at risk of developing cancer is likely to respond to treatment with a GREM1 antagonist, the method comprising measuring stromal expression of GREM1 in the patient, and thereby predicting whether or not the patient is likely to respond to treatment with the GREM1 antagonist. The above methods may further predict responsiveness to combined treatment with a GREM1 antagonist and a chemotherapeutic agent, or combined treatment comprising administration of a GREM1 antagonist and radiotherapy.

Predicted responsiveness in an individual to a given therapy means that the individual is expected to derive benefit, or a sufficient extent of benefit, from receiving the therapy. Predicted non-responsiveness in an individual to a therapy means that the individual is not expected to derive benefit, or a sufficient extent of benefit, from receiving the therapy. The method for predicting the response may be carried out before administration of the GREM1 antagonist or chemotherapeutic agent. The prediction may then be taken into account when selecting or recommending a suitable treatment for the individual. Alternatively, the method may be carried out after treatment with the therapy and used to monitor and predict the individual's response to treatment. Typically the

method is for predicting whether or not the individual will have a primary response to treatment with the therapy, i.e. whether or not the individual will respond when first receiving the treatment. In some cases the method is for predicting secondary non-responsiveness, i.e. whether or not an individual who initially responds to treatment will later stop responding to treatment or will respond less well to the treatment.

According to the present invention, increased stromal and/or epithelial level of GREM1 in an individual, as compared with a reference sample or reference level, indicates a positive diagnosis relating to the presence of cancer, for example that the individual has a cancer or a particular form of cancer or has more severe cancer. An increased stromal and/or epithelial level of GREM1 also indicates a negative prognosis, that is a poor predicted outcome for the individual, for example that the individual will not respond to a particular therapy, that an individual in remission from cancer will have a recurrence or that the individual is at increased risk of developing the cancer.

Conversely, a decreased or normal level of GREM1 indicates a negative diagnosis, for example that the individual does not have the cancer or has less severe cancer. A decreased level of GREM1 may indicate a positive prognosis, that is a good outcome for the patient, for example that the individual will respond to a particular therapy or that an individual in remission from the cancer will not have a recurrence or is not at increased risk of developing the disease or condition. For diagnosing whether or not an individual has the cancer, the reference sample or level typically represents a baseline level of GREM1 in an individual who does not have the relevant cancer, or who is suspected of having a cancer, but is subsequently confirmed to not have the cancer.

The method of diagnosis or prognosis may include selecting or recommending a suitable treatment for the individual, i.e. based on the diagnosis or prognosis. The selected or recommended treatment may then be administered to the individual. Thus, the above methods of detection, diagnosis, prognosis and prediction of responsiveness may further comprise a step of administering one or more prophylactic or therapeutic anti-cancer agents to the individual, or administering a cancer therapy, such as radiotherapy. The one or more agents typically comprise a GREM1 antagonist, any may further comprise an additional anti-cancer agent as described above.

In some cases, an overexpression of GREM1, as compared with a reference sample or reference level, indicates that the individual will respond to therapy with a GREM1

antagonist. A therapy including use of a GREM1 antagonist may then be selected or recommended, and may then further be administered to the individual. Similarly, a therapy comprising use of a GREM1 antagonist and an additional anti-cancer agent may be selected based on the overexpression of GREM1.

In other cases, a decreased or normal level of GREM1, as compared with a reference sample or reference level, indicates that the individual will not respond to therapy with a GREM1 antagonist. A GREM1 antagonist is then not administered to the individual. Further, a therapeutic treatment other than GREM1 antagonist may be selected or recommended for treatment of the individual, and may then further be administered to the individual.

In all aspects of the invention, an individual having cancer (e.g. colorectal cancer or multiple myeloma or breast cancer) or an individual suspected of having the disease or condition and/or an individual at risk of developing the disease or condition may be selected for treatment or identified. For example, the individual may not have been formally diagnosed but may be suspected of having the disease or condition because of the presence of one or more symptoms. The individual may be considered at risk of developing cancer if they have one or more risk factors associated with cancer and/or one or more predispositions which increase their susceptibility to cancer. Risk factors in relation to colorectal cancer include SNPs as described above and for example in Tomlinson et al, PLos Genet, 2011, discussed supra, and inherited genetic mutations such as mutation or mutations in the GREM1 encoding gene, or any other mutation affecting expression of the GREM1 gene, including the mutation causative of HMPS.

The following Examples illustrate the invention.

Examples

Example 1- Protein expression, purification, refolding and structure determination.

Protein expression and inclusion body preparation

A truncated human Gremlin-1 coding sequence (SEQ ID NO: 20), optimised for expression in *E.coli*, was cloned into a modified pET32a vector (Merck Millipore) using

BamHI/XhoI, generating a vector encoding the Gremlin sequence with an N-terminal 6His-TEV tag (pET-hGremlin1).

Expressed sequence:
*MGSSHHHHHHSSGENLYFQGS*AMPGEEVLESSQEALHVTERKYLKRDWCKTQPLK QTIHEEGCNSRTIINRFCYGQCNSFYIPRHIRKEEGSFQSCSFCKPKKFTTMMVTLNC PELQPPTKKKRVTRVKQCRCISIDLD; SEQ ID NO: 2 (with non-Gremlin residues of the 6His-TEV tag shown in italics). Sequence numbering based on UniProt O60565 & SEQ ID NO: 1.

The pET-hGremlin1 plasmid DNA was used to transform BL21(DE3) cells. A single ampicillin resistant colony was picked from a LB/Amp agar plate and used to inoculate a 100 ml starter culture of LB/Amp. After shaking (200 rpm) for 16 hr at 37 °C, 25 ml of the starter culture was used to inoculate 500 mL of 2xTY/Amp media. The culture was shaken (250 rpm) at 37 °C until an OD_{600} of 3 was achieved. Subsequently, the culture was supplemented with 20 mL of a MOPS + glycerol feed mix (1M MOPS pH 7.4, 40 % glycerol, 0.5 % $MgSO_4$, 0.42 % $MgCl_2$), induced with 300 µM IPTG and further incubated at 17 °C, 180 rpm for 16 hours. Cells were harvested in a centrifuge (4,000 g for 20 minutes at 4 °C).

Cell pellets were resuspended in Lysis Buffer (PBS pH 7.4, 0.35 mg/ml lysozyme, 10 µg/ml DNase and 3 mM $MgCl_2$) at 4 °C and the insoluble fraction was harvested by centrifugation at 3,500 g for 30 minutes at 4 °C. Pelleted inclusion bodies were washed three times by resuspending in wash buffer (50 mM Tris, 500 mM NaCl, 0.5 % Triton X-100, pH 8.0), followed by centrifugation at 21,000 g for 15 minutes. An additional two washes were performed using wash buffer without Triton X-100.

Solubilisation

Inclusion bodies were resuspended in denaturing buffer (8 M Urea, 100 mM Tris, 1 mM EDTA, 10 mM $Na_2S_4O_6$ and 100 mM Na_2SO_3, pH 8.5), stirred for 16 hrs at room-temperature and clarified by centrifugation at 21,000 g for 15 minutes.

Pre-refolding purification

The solubilized inclusion bodies were loaded onto a Sephacryl S-200 26/60 column (120 mL) equilibrated in 8 M Urea, 50 mM MES, 200 mM NaCl, 1 mM EDTA, pH 6.0. Fractions containing Gremlin-1 protein were diluted with 6 M Urea, 20 mM MES, pH 6.0 and loaded onto HiTrap SP HP cation exchange columns and eluted with a 1 M NaCl gradient over 10 column volumes (10 CVs). Fractions containing purified, denatured hGremlin-1 protein were pooled.

Refolding

Denatured purified Gremlin-1 protein was added drop-wise to re-folding buffer (50 mM Tris, pH 8.5, 150 mM NaCl, 5 mM GSH and 5 mM GSSG, 0.5 mM Cysteine, 5 mM EDTA, 0.5 M Arginine) to a final concentration of 0.1 mg/ml and incubated at 4 °C with constant stirring for 5 days. After 5 days the Gremlin-1 protein was dialysed against 20 mM HEPES, 100 mM NaCl, pH 7.5.

Following dialysis protein was applied to heparin HiTrap column and eluted using a gradient of 0-100 % heparin elution buffer (20 mM HEPES, 1 M NaCl, pH 7.5) over 20 CV. Correctly folded protein eluted at 1 M NaCl whereas any misfolded protein eluted at lower salt concentrations.

Protein eluting at 1 M NaCl was concentrated and purified further on a S75 26/60 column equilibrated with 20 mM Hepes, pH 7.5, 1 M NaCl.

Protein was characterised by SDS PAGE (shift in gel), demonstrated to have the expected molecular weight and correct arrangement of disulphide bonds using liquid chromatography mass spectrometry (LC-MS) and to be active in a cell assay (ID1 reporter assay).

Gremlin 1 structure determination

Gremlin 1 protein crystals were grown using the hanging-drop method by mixing a solution of Gremlin 1 at 6.6 mg/ml and 0.1 M citric acid at pH 4, 1 M lithium chloride and 27 % polyethylene glycol (PEG) 6000 in a 1:1 ratio. Before data collection, crystals were cryo-protected by adding 20 % glycerol to the crystallization buffer. Diffraction data were

collected at the Diamond Light Source and were processed using XDS (Kabsch, Wolfgang (2010) Acta Crystallographica Section D 66, 125—132). Diffraction data statistics are summarized in the table below:

Table 2: Diffraction data statistics

Diffraction Statistics	
Wavelength (Å)	0.97949
Space group	C2
Cell dimensions	a= 84.55 Å, b=107.22 Å, c=77.09 Å; α=90.00°, β=120.43°, γ=90.00°
Resolution range* (Å)	26.19-2.72 (2.79-2.72)
Completeness (%)	98.5 (99.0)
Multiplicity	3.4 (3.4)
I/sigma	9.6 (2.0)
Rmerge	0.095 (0.622)
Refinement Statistics	
Resolution Range (Å)	26.19-2.72
R_{cryst}	0.24
R_{free}	0.29
R.m.s.d. bonds (Å)**	0.013
R.m.s.d. angles (°)	1.782

*values in parenthesis correspond to the highest resolution shell

**r.m.s.d root mean square deviation

Gremlin-1 structure was solved by molecular replacement using Phaser (McCoy *et al*, J Appl Cryst (2007), 40, 658-674) and a Gremlin-1 model available from proprietary Gremlin-1 / Fab complex coordinates. The resultant model of Gremlin-1 contained four copies of Gremlin 1 monomer organised as two dimers. Model corrections were made with Coot (Emsley *et al* Acta Crystallographica Section D: Biological Crystallography 66 (4), 486-501) and coordinates were refined using Refmac (Murshudov *et al* REFMAC5 for the refinement of macromolecular crystal structures. Acta Crystallographica Section D:

Biological Crystallography. 2011;67(Pt 4):355-367). Final coordinates were validated with Molprobity (Chen et al. (2010) MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallographica D66:12-21). A summary of model refinement statistics is shown in Table 2 above. Full structural data for Gremlin-1 crystallography is provided in Table 1 (Figure 1) of PCT/EP2017/083650 filed 19 December 2017, incorporated herein by reference.

Example 2 – BMP Binding residues on Gremlin-1

As discussed above, Gremlin-1 belongs to the bone morphogenic protein (BMP) antagonist protein family within a sub-group known as the DAN family. Within the DAN family, Gremlin-1 shares greatest homology with Gremlin-2 (PRDC).

The 2.7 Å human Gremlin-1 structure resolved in Example 1 shares many features in common with the published mouse Gremlin-2 structure (Nolan *et al* (2013), Structure, 21, 1417–1429). The overall fold is very similar, with two copies of Gremlin-1 forming an antiparallel, non-covalent dimer, arranged in an arch. Each monomer adopts the characteristic finger-wrist-finger arrangement with a cystine-knot motif towards the wrist end, opposite the fingers. Sequence identity between the proteins is 52 % rising to 67 % within the sequence visible in the two structures. The most highly conserved region lies in the extensive dimer interface where all the key contact residues are 100 % conserved.

Residues involved in BMP's 2, 4 & 7 binding to mouse Gremlin-2 (PRDC) and DAN (NBL1) have been identified using mutagenesis (Nolan *et al* (2013), Structure, 21, 1417–1429 and Nolan *et al* (2014) J. Biol. Chem. 290, 4759-4771). The predicted BMP binding epitope encompasses a hydrophobic patch spanning across both monomers on the convex surface of the dimer. Six residues were identified by mutagenesis; Trp72, Phe96, Tyr98, Phe104, Tyr105 & Phe117 and are 100 % conserved in human Gremlin-1 (numbering based on the mouse Gremlin-2 sequence). The degree of homology extends to the positioning of the side chains which adopt an identical conformation in both proteins.

The amino acid numbering used in the Gremlin PDB file matches the numbering in the

published mouse Gremlin-2 structure based on a structural alignment. This enables like for like comparison of amino acids when describing the structures. However, for clarity the key residues identified as playing a role in BMP binding are shown below with numbering based on the PDB file and UniProt file of SEQ ID NO: 1 in brackets:

Trp72(93), Phe96(117), Tyr98(119), Phe104(125), Tyr105(126) & Phe117(138).

In both mouse Gremlin-2 and human Gremlin-1 the hydrophobic BMP binding epitope is partially buried by an alpha helix formed by the N-terminal residues of each protein. A model of BMP binding has been proposed whereby the N-terminus can flex, exposing the full BMP binding interface (Nolan *et al* (2013), Structure, 21, 1417–1429). In the present analysis, the N-terminal residues were removed from the human Gremlin-1 and mouse Gremlin-2 structures before rendering a surface to reveal the similarity of the BMP binding faces on each protein.

The literature only describes mutagenesis of six resides that have an effect on BMP binding. It is possible that the actual BMP epitope covers a larger surface area, encompassing neighbouring amino acids. By highlighting all residues, within 6Å of those mutated, on the surface of Gremlin-1, a larger region of Gremlin-1 is revealed that could potentially be targeted by a therapeutic. This more extensive region encompasses the following amino acids of human Gremlin-1:

Asp92-Leu99
Arg116-His130
Ser137-Ser142
Cys176-Cys178
(Numbering based on SEQ ID NO: 1)

By combining published information with the crystal structure information of human Gremlin-1, regions of human Gremlin-1 that offer themselves as a potential route for therapeutic intervention blocking its interaction with BMP's have been identified.

Example 3 – Hek Id1 reporter gene assay

Background

The Hek Id1 reporter gene assay uses Clone 12 Hek293-Id1 reporter cells. This cell line was stably transfected with Id1 transcription factor. Id1 is a transcription factor in the BMP signalling pathway. Gremlin is known to bind BMPs prevent binding to their receptors reducing the luciferase signal from the reporter gene. Therefore, using this reporter assay, it is possible to screen anti-Gremlin antibodies and see if there are any that block the interaction of Gremlin with BMPs. A restoration of the luciferase signal is seen in these cells if there is a blocking of this interaction.

Method

Clone 12 cells were cultured in DMEM containing 10 % FCS, 1x L-Glutamine & 1x NEAA. Cells are also grown in the presence of Hygromycin B (200 µg/ml) to ensure cells do not lose Id1 gene expression. Cells were assayed in DMEM containing 0.5 % FCS, 1x L-Glutamine & 1x NEAA. Hygromycin B is not needed for the short time that the cells are in the assay.

The cells were washed in PBS, lifted off using cell dissociation buffer, spun and counted before being seeded at 5x104/well in 70 µl (Density of $7.14x10^5$/ml). Plates used were white, opaque Poly-D-Lysine coated 96-well sterile. Cells go in incubator for about 3-4 hours to settle down. BMP heterodimers were reconstituted to 200 µg/ml in 4 mM HCL. BMP was diluted to 10 µg/ml in assay media using a glass vial to give a new working stock.

In a polypropylene plate, Gremlin-1 was diluted 1:2 for an 8 point dose response curve with a top final dose of 1 µg/ml.

An additional volume of 20 µl media was added per well and plates were incubated at 37 °C for 45 mins.

BMP prepared at 100x was added to all wells except wells containing cells only. All wells are made up to 60 µl with assay medium and incubated for a further 45 mins at 37 °C.

Post incubation, 30 µl of sample was transferred per well of assay plate and incubated for 20-24 hours before measuring luminescence signal.

Cell Steady Glo was thawed in advance at room temperature. Assay plates were cooled to room temperature for about 10-15 mins before adding the reagent. Luciferase signal was detected by addition of cell steady glo reagent (100 µl) for 20 minutes on shaker at room temperature and measuring luminescence using cell titre glo protocol on Synergy 2.

The maximum signal was generated from wells containing BMP and the minimum signal was generated from the wells containing cells only.

Results

Gremlin-1 full length and truncated forms were tested in the Hek-Id1 reporter gene assay to confirm the blocking activity against BMP4/7.

The percentage of inhibition from dose response assays was calculated based on the maximum and minimum signals in the assay and the data fitted using 4 parameter logistical fit. The IC_{50} was calculated based on the inflexion point of the curve.

Table 3: Potency results for full length Gremlin-1 and truncated Gremlin-1 in the Hek-Id1 reporter gene assay.

Hek-Id1 Reporter gene assay	**N**	**Geometric mean (nM)**	**95% CI (or range where N=<4)**
Gremlin 1 Full length	2	1.6	1.3-1.9
Gremlin 1 truncated	2	1.7	1.1-2.5

Conclusion

Gremlin 1 was able to inhibit the BMP 4/7 signalling in the Hek-Id1 reporter gene assay.

Example 4 – Production of anti-Gremlin-1 antibodies

Anti-Gremlin-1 antibodies were derived by immunisation using purified gremlin-1 as described in Example 1, and by library panning. The library was generated in-house as a naïve human library with the V-regions amplified from blood donations.

Immunisation yielded 26 distinct antibodies binding Gremlin-1 from the first round of immunisation. These antibodies were scaled up and purified for testing in screening assays.

25 human and mouse cross-reactive antibodies from the library were panned using recombinant human Gremlin from R&D Systems. 10 antibodies were selected for scale up and purified as scFvs for testing in the screening assays.

Example 5 – Screening of anti-Gremlin-1 antibodies

Antibodies were screened using the Hek-Id1 reporter gene assay described in Example 3 and by measuring SMAD phosphorylation. SMAD1, 5 and 8 are phosphorylated upon BMP signalling. Inhibitors of Gremlin-1 therefore increase SMAD phosphorylation.

SMAD phosphorylation assays were conducted on A549 cells or on human lung fibroblasts. Phosphorylation levels were determined using MSD.

Results

In the Hek-Id1 reporter gene assay, there were no apparent hits with the immunisation derived antibodies (with a 10 fold excess of antibody tested against a BMP4/7 heterodimer). Results are shown in Figure 1.

In contrast, a number of library derived antibodies were capable of restoring signal in the Hek-Id1 reporter gene assay (50-fold excess of antibodies with a 50 % gremlin dose) (Figure 2). Of these, Ab2416 and Ab2417 contained high levels of endotoxin. Ab7326 maintained blocking ability at a 10-fold excess and 80 % inhibition Gremlin-1

concentration.

Additional results are presented in Figures 3A (human gremlin) and 3B (mouse Gremlin). These Figures show titrations of Ab7326 (labelled as PB376) up to 15 nM. Ab7326 was shown to restore signalling of BMP when blocked by either human (IC_{50} of 1.3 nM) or mouse (IC_{50} of 0.2 nM Gremlin). The antibody functions both as a human and mouse IgG1.

Sequences of the mouse and human full length IgG1 are presented below. In order to synthesise the mouse and human full length IgG1 proteins, the Ab7326 variable regions derived from the library were re-cloned into vectors comprising the appropriate antibody constant domains.

Because Ab7326 came from a naïve human library, where Abs are cloned as scFvs, in order to re-clone the 7326 variable regions as full length Abs or Fabs, it was necessary to PCR amplify the VH and VK using pools of primers/degenerate primers. The amplified PCR products were then digested and cloned simultaneously into mouse and human vectors. As the VH and VK were amplified by pools of primers/degenerate primers, two variant forms of the products were obtained, differing by a single amino acid residue derived from subtly different primers annealing during the PCR process.

The two variant forms of heavy chain variable region differed by a single amino acid at position 6, and the two variant forms of the light chain variable region differed by a single amino acid at position 7, as shown below:

- Heavy chain variable region variant 1 has glutamic acid (E) at position 6.
- Heavy chain variable region variant 2 has glutamine (Q) at position 6.
- Light chain variable region variant 1 has serine (S) at position 7.
- Light chain variable region variant 2 has threonine (T) at position 7.

Mouse full length IgG1 – heavy chain variant 1 (SEQ ID NO: 14)

QVQLVESGAE VKKPGATVKI SCKVS*GYTFT* **DYYMH**WVQQA PGKGLEWMG**L**
VDPEDGETIY AEKFQGRVTI TADTSTDTAY MELSSLRSED TAVYYCAT**DA**

RGSGSYYPNH FDYWGQGTLV TVSSAKTTPP SVYPLAPGSA AQTNSMVTLG
CLVKGYFPEP VTVTWNSGSL SSGVHTFPAV LQSDLYTLSS SVTVPSSTWP
SETVTCNVAH PASSTKVDKK IVPRDCGCKP CICTVPEVSS VFIFPPKPKD
VLTITLTPKV TCVVVDISKD DPEVQFSWFV DDVEVHTAQT QPREEQFNST
FRSVSELPIM HQDWLNGKEF KCRVNSAAFP APIEKTISKT KGRPKAPQVY
TIPPPKEQMA KDKVSLTCMI TDFFPEDITV EWQWNGQPAE NYKNTQPIMD
TDGSYFVYSK LNVQKSNWEA GNTFTCSVLH EGLHNHHTEK SLSHSPGK

Mouse full length IgG1 – light chain variant 1 (SEQ ID NO: 15)

DIVMTQSPDS LAVSLGERAT INC**KSSQSVL YSSNNKNYLA** WYQQKPGQPP
KLLIY**WASTR ES**GVPDRFSG SGSGTDFTLT INSLQAEDVA VYFC**QQYYDT**
PTFGQGTRLE IKRTDAAPTV SIFPPSSEQL TSGGASVVCF LNNFYPKDIN
VKWKIDGSER QNGVLNSWTD QDSKDSTYSM SSTLTLTKDE YERHNSYTCE
ATHKTSTSPI VKSFNRNEC

Mouse full length IgG1 – heavy chain variant 2 (SEQ ID NO: 28)

QVQLVQSGAE VKKPGATVKI SCKVS*GYTFT* **DYYMH**WVQQA PGKGLEWMG**L**
VDPEDGETIY AEKFQGRVTI TADTSTDTAY MELSSLRSED TAVYYCAT**DA**
RGSGSYYPNH FDYWGQGTLV TVSSAKTTPP SVYPLAPGSA AQTNSMVTLG
CLVKGYFPEP VTVTWNSGSL SSGVHTFPAV LQSDLYTLSS SVTVPSSTWP
SETVTCNVAH PASSTKVDKK IVPRDCGCKP CICTVPEVSS VFIFPPKPKD
VLTITLTPKV TCVVVDISKD DPEVQFSWFV DDVEVHTAQT QPREEQFNST
FRSVSELPIM HQDWLNGKEF KCRVNSAAFP APIEKTISKT KGRPKAPQVY
TIPPPKEQMA KDKVSLTCMI TDFFPEDITV EWQWNGQPAE NYKNTQPIMD
TDGSYFVYSK LNVQKSNWEA GNTFTCSVLH EGLHNHHTEK SLSHSPGK

Mouse full length IgG1 – light chain variant 2 (SEQ ID NO: 29)

DIVMTQTPDS LAVSLGERAT INC**KSSQSVL YSSNNKNYLA** WYQQKPGQPP
KLLIY**WASTR ES**GVPDRFSG SGSGTDFTLT INSLQAEDVA VYFC**QQYYDT**
PTFGQGTRLE IKRTDAAPTV SIFPPSSEQL TSGGASVVCF LNNFYPKDIN
VKWKIDGSER QNGVLNSWTD QDSKDSTYSM SSTLTLTKDE YERHNSYTCE
ATHKTSTSPI VKSFNRNEC

Human full length IgG1 – heavy chain variant 1 (SEQ ID NO: 30)

QVQLVESGAE VKKPGATVKI SCKVS*GYTFT* **DYYMH**WVQQA PGKGLEWMG**L**
VDPEDGETIY AEKFQGRVTI TADTSTDTAY MELSSLRSED TAVYYCAT**DA**
RGSGSYYPNH FDYWGQGTLV TVSSASTKGP SVFPLAPSSK STSGGTAALG
CLVKDYFPEP VTVSWNSGAL TSGVHTFPAV LQSSGLYSLS SVVTVPSSSL
GTQTYICNVN HKPSNTKVDK KVEPKSCDKT HTCPPCPAPE LLGGPSVFLF
PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE

EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP
REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT
TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL
SPGK

Human full length IgG1 – light chain variant 1 (SEQ ID NO: 31)

DIVMTQSPDS LAVSLGERAT INC**KSSQSVL YSSNNKNYLA** WYQQKPGQPP
KLLIY**WASTR ES**GVPDRFSG SGSGTDFTLT INSLQAEDVA VYFC**QQYYDT**
PTFGQGTRLE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK
VQWKVDNALQ SGNSQESVTE QDSKDSTYSL SSTLTLSKAD YEKHKVYACE
VTHQGLSSPV TKSFNRGEC

Human full length IgG1 – heavy chain variant 2 (SEQ ID NO: 16)

QVQLVQSGAE VKKPGATVKI SCKVS*GYTFT* **DYYMH**WVQQA PGKGLEWMG**L**
VDPEDGETIY AEKFQGRVTI TADTSTDTAY MELSSLRSED TAVYYCAT**DA**
RGSGSYYPNH FDYWGQGTLV TVSSASTKGP SVFPLAPSSK STSGGTAALG
CLVKDYFPEP VTVSWNSGAL TSGVHTFPAV LQSSGLYSLS SVVTVPSSSL
GTQTYICNVN HKPSNTKVDK KVEPKSCDKT HTCPPCPAPE LLGGPSVFLF
PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE
EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP
REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT
TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL
SPGK

Human full length IgG1 – light chain variant 2 (SEQ ID NO: 17)

DIVMTQTPDS LAVSLGERAT INC**KSSQSVL YSSNNKNYLA** WYQQKPGQPP
KLLIY**WASTR ES**GVPDRFSG SGSGTDFTLT INSLQAEDVA VYFC**QQYYDT**
PTFGQGTRLE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK
VQWKVDNALQ SGNSQESVTE QDSKDSTYSL SSTLTLSKAD YEKHKVYACE
VTHQGLSSPV TKSFNRGEC

Antibody CDRs were determined using the Kabat method (highlighted in bold in the above sequences). Additional HCDR1 residues using the Chothia definition are in italics. Constant region sequences are underlined.

Restoration of p-SMAD signalling with anti-Gremlin 1 antibodies is shown in Table 4 below.

Table 4: Restoration of p-SMAD signalling

	2417	2418	2419	2481	2482	2483	**2484**	**7326**	8427
BMP 2 50ng/ml	**109.1% +/- 2.8%**	58.2% +/- 1.9%	32.6% +/- 1.4%	40.4% +/- 0.6%	35.3% +/- 0.8%	43.1% +/- 2.1%	**104.0% +/- 2.7%**	**107.2% +/- 3.5%**	51.3% +/- 1.4%
BMP 4 25ng/ml	**109.6% +/- 3.0%**	71.3% +/- 3.1%	31.7% +/- 1.2%	60.1% +/- 2.2%	54.4% +/- 1.3%	72.5% +/- 2.1%	**105.2% +/- 3.3%**	**110.0% +/- 3.8%**	78.2% +/- 2.5%
BMP 7 200 ng/ml	**111.5% +/- 3.8%**	**99.5% +/- 3.2%**	53.8% +/- 3.4%	64.4% +/- 1.3%	52.3% +/- 1.1%	66.2% +/- 1.2%	**105.2% +/- 4.3%**	**108.0% +/- 3.2%**	72.6% +/- 2.5%
BMP-2/7 50ng/ml	**119.3% +/- 2.6%**	78.6% +/- 3.6%	50.8% +/- 2.7%	53.7% +/- 3.1%	47.6% +/- 1.5%	56.1% +/- 2.5%	**120.4% +/- 4.4%**	**128.5% +/- 2.9%**	62.8% +/- 2.5%
BMP4/7 50ng/ml	**113.7% +/- 3.1%**	78.0% +/- 4.0%	61.4% +/- 4.0%	48.3% +/- 2.1%	41.7% +/- 1.7%	50.8% +/- 1.7%	**112.4% +/- 2.5%**	**127.0% +/- 3.1%**	63.3% +/- 2.1%

Results are shown as a percentage of SMAD phosphorylation by BMP alone (control BMP). Experiments were performed using lung fibroblasts from idiopathic pulmonary fibrosis patients. rhGremlin-1 and the anti-Gremlin-1 antibodies were preincubated for 45 minutes at room temperature. rhGremlin-1 and the anti-Gremlin-1 antibodies were then added with BMP to the cells for 30 minutes.

Table 5 then shows further results in the SMAD phosphorylation assay, where displacement of BMP-2 or BMP4/7 from Gremlin 1-BMP complexes by anti-Gremlin-1 antibodies was investigated. Experiments were again performed using lung fibroblasts from idiopathic pulmonary fibrosis patients. rhBMP-2 or rhBMP 4/7 were preincubated with rhGremlin-1 for 1 hour at room temperature. The BMP-2- or BMP4/7-Gremlin-1 complexes were incubated with different concentrations of the anti-Gremlin-1 antibodies overnight at 4 °C. Antibody concentrations represent the final concentration on the plate.

Table 5: Displacement of BMP-2 or BMP4/7 from Gremlin 1-BMP complexes by anti-Gremlin-1 antibodies

		81.3 µg/ml	40.6 µg/ml	20.3 µg/ml	10.2 µg/ml	5.1 µg/ml	2.55 µg/ml	1.27 µg/ml	0.63 µg/ml
2484	**BMP 2 50ng/ml**	100.3% +/- 3.5%	98.8% +/- 2.7%	97.0% +/- 2.9%	93.5% +/- 2.6%	86.4% +/- 2.0%	79.9% +/- 1.9%	66.5% +/- 2.8%	54.8% +/- 0.3%
2484	**BMP4/7 50ng/ml**	136.4% +/- 4.2%	133.2% +/- 1.0%	121.4% +/- 1.4%	108.1% +/- 4.9%	86.6% +/- 4.4%	74.7% +/- 2.2%	65.8% +/- 0.6%	60.7% +/- 1.5%
7326	**BMP 2 50ng/ml**	103.7% +/- 1.1%	101.5% +/- 2.4%	99.4% +/- 3.8%	103.8% +/- 2.4%	100.3% +/- 2.2%	103.2% +/- 4.3%	102.8% +/- 2.8%	97.0% +/- 2.9%
7326	**BMP4/7 50ng/ml**	133.7% +/- 0.8%	132.3% +/- 1.8%	130.3% +/- 4.2%	125.6% +/- 10.0%	121.4% +/- 4.2%	120.9% +/- 3.3%	111.1% +/- 2.3%	102.0% +/- 4.5%

The results shown in Table 5 demonstrate that Ab7326 can displace already complexed BMP-2 or BMP4/7 from Gremlin 1-BMP complexes. Ab7326 can achieve this displacement at much lower concentrations that the comparison antibody 2484. This provides evidence that Ab7326 is an allosteric inhibitor, consistent with our finding that the binding site for Ab7326 is distal from the known BMP binding regions on gremlin-1. Thus Ab7326 is able to access the allosteric binding site even when BMP is complexed to gremlin-1, resulting in significantly improved inhibition of gremlin activity.

Example 6 – Obtaining the crystal structure of Gremlin-1 in complex with the 7326 Fab

The crystal structure of human Gremlin-1 in complex with Ab7326 Fab was solved at a resolution of 2.1 Å. Fab sequences are shown below:

Heavy chain: SEQ ID NO: 18

QVQLVESGAEVKKPGATVKISCKVSGYTFTDYYMHWVQQAPGKGLEWMGLVDPEDGETIYA
EKFQGRVTITADTSTDTAYMELSSLRSEDTAVYYCATDARGSGSYYPNHFDYWGQGTLVTV

SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC

Light chain: SEQ ID NO: 19

DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRE
SGVPDRFSGSGSGTDFTLTINSLQAEDVAVYFCQQYYDTPTFGQGTRLEIKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST
LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

The CCP4 software NCONT was then used to identify all contacts at 4 Å between Gremlin-1 and the Fab. The following residues were identified: Ile131, Lys147, Lys148, Phe149, Thr150, Thr151, Arg169, Lys174 and Gln175 (numbering based on the UniProt Sequence of SEQ ID NO: 1 (numbered as Ile110, Lys126, Lys127, Phe128, Thr129, Thr130, Arg148, Lys153 and Gln154 in the structure file which matches the numbering of mouse Gremlin-2).

Figure 4 shows structural models of the Gremlin-Fab complex, with the Fab epitope residues shown relative to the BMP binding regions.

Ab7326 is an inhibitory antibody which acts allosterically, i.e. it binds away from the BMP binding regions.

Example 7 – Affinity measurements for binding of anti-Gremlin-1 antibody Ab7326 to Gremlin-1.

Method

The affinity of anti-Gremlin mIgG for human Gremlin 1 was determined by biamolecular interaction analysis using surface plasmon resonance (SPR) technology on a Biacore T200 system, GE Healthcare Bio-Sciences AB. Anti-Gremlin mIgG was captured by an immobilised anti-mouse Fc surface and Gremlin 1 was titrated over the captured mIgG. The capture ligand (affinipure $F(ab')_2$ fragment of goat anti-mouse IgG, Fc fragment specific, 115-006-071, Jackson ImmunoResearch Inc.) was immobilised at 50µg/ml in 10mM NaAc, pH 5.0 on flow cell 2 of a CM4 Sensor Chip via amine coupling chemistry,

using 600s activation and deactivation injections, to a level of ~1600 response units (RU). HBS-EP+ buffer (0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.05 % Surfactant P20) was used as the running buffer with a flow rate of 10 µl/min. A reference surface was prepared on flow cell 1 by activating and deactivating the surface as for flow cell 2 but omitting the capture ligand.

The assay buffer was HBS-EP+ plus an extra 150 mM NaCl to give a final NaCl concentration of 300 mM plus 1% CMD40. A 60 s injection of anti-Gremlin mIgG (at 5µg/ml in running buffer) was passed over flow cells 1 and 2 to give a capture level of approximately 100 RU on the immobilised anti-mouse IgG, Fc surface. Recombinant human Gremlin 1 was titrated in running buffer from 5 nM (using 2-fold dilutions) and injected over flow cells 1 and 2 at a flow rate of 30 µl/min for 3min followed by a 5min dissociation phase. A buffer only control was also included. The surface was regenerated at a flow rate of 10 µl/min by a 60 s injection of 50 mM HCl, a 30 s injection of 5 mM NaOH and a 30 s injection of 50 mM HCl.

The kinetic data was determined using Biacore T200 evaluation software. The affinity measurements were made at 25°C.

Results

Binding affinity, taken as the average K_D value for 5 determinations, was found to be below 100 pM.

Example 8 - Anti-Grem1 antibody inhibited mouse organoid culture

Materials and Methods

Mouse procedures. All procedures were carried out in accordance to Home Office UK regulations and the Animals (Scientific Procedures) Act 1986. All mice were housed at the animal unit at Functional Genomics Facility, Wellcome Trust Centre for Human Genetics, Oxford University. All strains used in this study were maintained on C57Bl/6J background for ≥ 6 generations. Genotyping protocols for the *Apc*^{Min} (1) and *Vil1-Grem1* (2), Apc$^{fl/fl}$

(3) and VillinCreERT2 (4) mice have previously been reported. To generate Kaplan-Meier data, mice were sacrificed when reached humane-end points (exhibited anaemia, hunching and inactivity). Anti-Grem1 antibody (UCB Ab7326 mouse IgG1, as also used in all subsequent mouse experiments) or mouse IgG1 control Ab101.4 antibody (UCB) were administered at a dose of 10 mg/kg or 30 mg/kg weekly or bi-weekly subcutaneous injections. For long-term treatment cohorts, a 30 mg/kg dose was delivered weekly in Vil-Grem1 and Apc^{Min} mice from 6-weeks of age and the Vil1-Grem1/ Apc^{Min} cohort treatment commenced at 3-weeks of age, twice weekly for 6 weeks, once weekly thereafter.

Tissue preparation and histology. Mice were sacrificed at pre-defined time points or when showing symptoms of intestinal polyps (anaemia, hunching) by cervical dislocation. The intestinal tract was removed immediately and divided into small intestine (proximal/SB1, middle/SB2 and distal/SB3) and large intestine. The intestines were opened longitudinally, using a gut preparation apparatus (5), washed in PBS, fixed overnight in 10% neutral buffered formalin (NBF). For visualization of polyps, gut preparations were stained with 0.2% methylene blue for 10 s and viewed with the aid of a light box. Specimens of 10% formalin-fixed tissue were embedded in paraffin and then sectioned at 4 µm. Fixed specimens were embedded and H&E stained following standard protocols.

Immunohistochemistry. Formalin-fixed, paraffin-embedded tissue sections (4 µm) were de-waxed in xylene and rehydrated through graded alcohols to water. Endogenous peroxidase was blocked using 1.6% H_2O_2 for 20 min. For antigen retrieval, sections were pressure cooked in 10 mmol/L citrate buffer (pH 6.0) for 5 min. Sections were blocked with 10% serum for 30 min. Slides were incubated with primary antibody for 2 h. The following antibodies have been used in this study; Cytokeratin 20 (Abcam, ab118574, 1:200), EphB2 (R and D, AF467, 1:125), Ki67 (CST, 122025, 1:500), Lysozyme (DAKO, EC 3.2.1.17, 1:500) and Sox9 (Millipore, ab5535, 1:1000). Appropriate secondary antibodies were applied for 1 h at room temperature. Sections were then incubated in ABC (Vector labs) for 30 min. DAB solution was applied for 2–5 min and development of the colour reaction was monitored microscopically. Slides were counterstained with haematoxylin, dehydrated, cleared and then mounted.

***In situ* hybridisation (ISH).** 4 μm sections were prepared using DEPC (Sigma) treated H_2O. *In situ* hybridisation was carried out using *Grem1* (314741) (Advanced Cell Diagnostics) probe and the RNAscope 2.5 HD Detection Kit (Advanced Cell Diagnostics) following manufacturer's instructions.

Culture of mouse intestinal crypts. Mouse intestinal crypts were isolated and cultured as described by Sato *et al* (6). In brief, crypts were isolated, resuspended in Matrigel (BD Biosciences) and plated out in 24-well plates. The basal culture medium (advanced Dulbecco's modified Eagle medium/F12 supplemented with penicillin/streptomycin, 10 mmol/L HEPES, Glutamax, 1x N2, 1x B27 (all from Invitrogen), and 1 mmol/L *N*-acetylcysteine (Sigma)) was overlaid containing the following growth factors; Epidermal Growth Factor at 50 ng/ml (Life Technologies), Gremlin1 at 100 ng/ml (R and D), R-spondin1 at 500 ng/ml (R and D) [EGR media]. Anti-Grem1 antibody (UCB) was used at a concentration of 1 mg/ml. The media was changed every two days.

Western blotting. Isolation of colon epithelium cells was carried out by incubating a 2 cm long piece of tissue with 30 mM EDTA for 2 h with agitation at 4°C. Pelleted epithelial cells were lysed RIPA lysis buffer with the addition of protease inhibitors (Complete Mini, Roche) and phosphatase inhibitors (PhosSTOP, Roche). All lysates were quantified using the BCA assay (Thermo Scientific), 35mg was diluted in an appropriate amount of 4x loading dye (Invitrogen) and denatured at 95°C for 5 minutes. Western blotting was done with the NuPAGE Gel system, (Invitrogen) according to manufacturer's protocol. Briefly, denatured lysates were loaded onto a 4-12% gel and run at 100 V for at least 2 hours. The gels were transferred onto PVDF membrane (Immobilon P, Millipore) in a semi-dry tank (120 mA for at least 2 hours) and blocked by incubating for 1 hour at room temperature in TBS containing 10% milk (Marvel). The membranes were then incubated overnight in the appropriate primary antibody in TBS with 5% milk, pSmad1/5/8 (Cell Signalling, 9516S, 1:500), Actin (Santa Cruz, sc-47778, 1:2500). After washing, the membranes were incubated with HRP-conjugated secondary antibodies for 1 hour at room temperature. After further washes, the blots were incubated in ECL reagents (GE healthcare) and chemiluminescence was detected by chemiluminescence film (GE Healthcare).

Description of Results (see also Figure 5)

A mouse organoid culture was used to assess effects of an anti-GREM1 antibody. Successful intestinal epithelial organoid culture is dependent on media supplementation of exogenous BMP antagonist. Recombinant Noggin and Gremlin1 can be used interchangeably for this purpose, and intestinal crypt culture in the absence of one of these proteins does not proceed past day 4 of culture.

Approximately 60% of crypts formed organoids in media fully supplemented with recombinant epidermal growth factor (E), Grem1 (G) and R-Spondin1, (R) (EGR media). Culture success rate dropped to less than 2% at 7 days in media lacking recombinant Grem1 (ER media).

Addition of 1mg/ml of anti-Grem1 antibody to otherwise fully supplemented media (EGR+antibody) prevented successful intestinal organoid culture, consistent with an abrogation of the BMP antagonistic effects of Grem1 in fully supplemented media. These results were indistinguishable from Grem1 excluded media (ER).

Example 9 - Anti-Grem1 antibody treatment partially restores intestinal pSMAD1,5 signalling in treated Vil1-Grem1 animals (see also Figure 6)

Epithelial BMP activity can be measured by phosphorylation of the SMAD 1,5,8 intracellular signalling transducers.

In Vil1-Grem1 animals, aberrant epithelial expression of Grem1 initiates polyposis through promotion of an aberrant stem/progenitor cell phenotype in cells situated outside of the colonic crypt base. Vil1-Grem1 mice develop a profound pan intestinal polyposis from about 8 weeks of age, characterised by villus ectopic crypt formation, aberrant cell proliferation and initiation of villus dysplasia.

The aberrant epithelial Grem1 expression in Vil1-Grem1 mice suppressed physiological intestinal BMP pathway activity with reduction in detected pSMAD1,5 protein levels detected by Western blot.

Weekly subcutaneous anti-Grem1 antibody administration at a dose of 30 mg/kg but not at the lower dose of 10mg/kg, was able to restore intestinal epithelial pSMAD1,5 signalling in Vil1-Grem1 intestinal epithelium. Indicating functional antagonism of Grem1.

Example 10 - Anti-Grem1 antibody treatment abrogates Vil1-Grem1 pan-intestinal polyposis phenotype and restores normal cell fate determination (see Figures 7 and 30)

Twice weekly, subcutaneous administration of anti-Grem1 antibody (30 mg/kg) for a period of 6 weeks to the Vil1-Grem1 mice also resulted in a dose-dependent and highly significant abrogation of this pan-intestinal polyposis phenotype, alongside therapeutic near-normalization of the aberrant intestinal crypt-villus architecture that characterizes this animal model.

Immunohistochemical staining of treated animals showed reversal of villus ectopic crypt formation and deranged cell fate determination that characterised the untreated tissue (Figures 7 and 30).

Example 11 - Prolonged treatment with anti-Grem1 antibody in animal models with Grem1 initiated polyposis is safe and significantly prolongs animal lifespan (see Figures 8 and 31)

Animals were treated with twice weekly (*Vil1-Grem1;Apc^{Min}*) or weekly (*Vil1-Grem1*) with subcutaneous anti-Grem1 antibody at a dose of 30 mg/kg, initiated at 3 (*Vil1-Grem1;Apc^{Min}*) or 6 weeks of age (*Vil1-Grem1*).

Prolonged dosing of anti-Grem1 antibody in Grem1 initiated tumourigenesis models slowed polyp formation, reduces tumour burden and significantly prolongs animal lifespan in *Vil1-Grem1* animals. This tumour abrogating effect was consistent across lesions initiated by aberrant epithelial Grem1 expression, even in the aggressive *Vil1-Grem1;*

Apc^{Min} strain, where antibody treatment more than doubles animal lifespan (VG-min mean lifespan 46 days versus VG-min treated mean lifespan 108 days, p = 2.21 x10-6)

No consistent adverse events have been observed to date in animals treated for more than 400 days.

Example 12 - Pharmacological downregulation of Grem1 attenuates mutant Apc-driven tumourigenesis in *Apc*^{Min} mice (see Figures 9 and 32)

The effect of GREM1 antagonism was also investigated in a mouse model of sporadic cancer, caused by epithelial Apc inactivation. Epithelial-mesenchymal signalling crosstalk means that stromal cells are responsive to acute epithelial inactivation of Apc, with rapid upregulation of intestinal muscularis and propria Grem1 occurring just 5 days after epithelial cell inactivation of Apc in Villin-CreERT2;Apcfl/fl mice (figure 9A).

Long term treatment with anti-Grem1 antibody initiated at 6 weeks of age had a consistent effect on *Apc*^{Min} mouse polyp development, allowing prolonged survival of animals through reduced tumour burden. This work indicates that stromal upregulation of Grem1 may exacerbate mutant Apc-driven tumourigenesis and that anti-Grem1 therapy may be useful for treatment of non-Grem1 initiated tumourigenesis.

Example 13 - Grem1 is overexpressed in the Myeloma bone marrow microenvironment and can be targeted to reduce myeloma growth

This study demonstrates for the first time that Grem1 plays a role in multiple myeloma (MM) disease progression. Analysis of stromal cells derived from patient bone marrow (BM) trephine biopsies demonstrated a significant increase in Grem1 expression from the BM microenvironment during MM. In addition, the data presented here shows that increased levels of Grem1 promote the proliferation of MM PC, and that Grem1 can be targeted to significantly reduce MM tumour burden in a preclinical mouse model of MM. This represents the first evidence that Grem1 plays a role in MM disease progression, and as such represents a therapeutic target for the treatment of MM.

Methods

Culture of Myeloma Cell Lines: Unless otherwise stated, all tissue culture media contained 10% (v/v) FCS and additives (2 mM L-glutamine, 1 mM sodium pyruvate, 15 mM HEPES, 50 U/mL penicillin and 50 µg/mL streptomycin; all from Sigma-Aldrich, Sydney, Australia). Mouse 5TGM1 MM cells were maintained in Iscove's Modified Eagle's Medium supplemented with 20% (v/v) FCS and additives. We have previously modified the 5TGM1 cells (Dallas *et al.*, 1999) with a trimodality retroviral NES-TGL construct (Diamond *et al.*, 2009; Ponomarev *et al* (2004) encoding thymidine kinase, green fluorescent protein (GFP), and firefly luciferase (Noll *et al.*, 2014), and a new clonal subline was established that exhibits consistent bone tropism (Noll *et al.*, 2014; (Noll *et al.*, 2015). OP9 bone marrow stromal cells were maintained in Dulbecco's Modified Eagle Medium with 10% (v/v) FCS and additives. Co-culture of 5TGM1 cells and OP9 cells were maintained in Iscove's Modified Eagle's Medium supplemented with 20% (v/v) FCS and additives. Human MM cell lines RPMI-8226, U266, KMS-11 and H929 were all cultured in RPMI-1640 medium (Cheong *et al.*, 2015). All cell lines were maintained in a humidified environment at 37°C with 5% carbon dioxide.

Isolation of RNA from murine BM stroma: C57BL6/KaLwRij.Hsd mice were injected with $5x10^5$ 5TGM1.Bmx1 MM PCs, and tumour growth was established over 4 weeks (as previously described) (Noll *et al.*, 2014; Hewett *et al.,* 2017). Briefly, 5TGM1 cells were resuspended at 5×10^6 cells per ml in phosphate-buffered saline (PBS), and then 5×10^5 cells were injected into the tail vein of 6- to 8-week-old C57BL/KaLwRij mice. Growth of tumours was monitored by whole animal bioluminescent imaging (BLI) using a Xenogen IVIS 100 Imaging System (Caliper Life Sciences, Hopkinton, USA)) after intraperitoneal injection (i.p.) of 150 mg/kg of D-Luciferin (Biosynth, Raad, Switzerland). Tumour burden was quantitated using Living Image software. Mice with tumour burden and age-matched non-tumour controls were humanely culled as per SAHMRI ethics SAM165. Femurs and tibias for each mouse was isolated and flushed of marrow with PBS, 2% FCS, and 2 mM EDTA (PFE). Bones were crushed and digested in 3ml of 3mg/ml collagenase I for 2 hours at 37°C in a shaking incubator. Suspension was diluted to 10ml with PFE. Spun at 1400g for 5min to collect cells. Wash with 1xPFE and repeat spin. The

cell pellet and bone chips were resuspended in 1ml of TRIzol (Thermo Fisher Scientific Inc., Massachusetts, USA) - vortex and incubated on ice for 15min. The TRIzol was collected and processed for RNA extraction by chloroform/isopropanol precipitation (as previously described).

Assessment of Grem1 expression in human and murine stroma: Iliac crest trephines were collected, with informed consent, from randomly selected patients with symptomatic MM who presented at the Royal Adelaide Hospital (Adelaide, Australia), and from haematologically normal age-matched controls. All MM patients were newly diagnosed and had not had previous therapy. Ethical approval for this study was obtained from the Royal Adelaide Hospital institutional ethics review committee (ethics approval number #030206). Adherent stromal cells were expanded ex vivo from each trephine and cryopreserved after one further passage. Stromal samples were retrieved from storage in liquid nitrogen and cultured for 24hours prior to collection in TRIzol for downstream RNA extraction as described previously. RNA underwent DNase treatment with RQ1 DNase (Promega, Wisconsin, USA) prior to cDNA manufactured using SuperScriptIII (Thermo Fisher Scientific Inc., Massachusetts, USA) Quantitative PCR analysis of Grem1 expression in both human and murine samples was performed using RT2 SYBR-green master mix (Qiagen, Hilden, Germany) on a BioRad CFX Connect with normalisation to B-actin as the endogenous control using the standard curve method. The following primers were used: mouse Grem1: forward 5'-GCGCAAGTATCTGAAGCGAG-3' (SEQ ID NO: 38); reverse 5'-CGGTTGATGATAGTGCGGCT-3' (SEQ ID NO: 39), human Grem1: forward 5'-AGGCCCAGCACAATGACTCAG-3' (SEQ ID NO: 40); reverse 5'-GTCTCGCTTCAGGTATTTGCG-3' (SEQ ID NO: 41);
B-actin: forward 5'-GATCATTGCTCCTCCTGAGC-3' (SEQ ID NO: 42);
reverse 5'-GTCATAGTCCGCCTAGAAGCAT-3' (SEQ ID NO: 43).

Generation of a murine stromal Grem1-overexpressing cell line: The murine cDNA for Grem1 was isolated from a pCMV6-K^R vector kindly provided by the Gastrointestinal Cancer Biology Group, SAHMRI. The murine Grem1 sequence was excised by EcoR1 and Not1 restriction enzyme digest and cloned into the pLeGoiT2 vector. Following lentiviral

infection of OP9-GFP+ cells, cells positive for GFP and TdTomato were sorted by FACS. Transgene expression was confirmed by quantitative-PCR and Western blot.

Co-culture of MM PC and BM stroma: OP9 stromal cells were seeded in both a 6cm TC dish and 24-well plate at [cell density], respectively and allowed to adhere for 5 hours. 5TGM1 MM PC were suspended at $1x10^5$ cells/ml and added to the stromal cell cultures. Stromal cells were collected at 24, 48 and 72 hours post co-culture initiation. For contact co-culture the GFP+OP9 cells were isolated by FACS from the GFP negative 5TGM1-parental MM PCs to obtain a pure stromal population for analysis. For non-contact co-culture, 5TGM1.Bmx1 PCs were separated from the OP9 cells using a 4μm Transwell for the duration of the co-culture. Human MM cell lines RPMI-8226, U266, KMS-11 and H929 were each cultured with 3 primary human BM stromal samples isolated from haematopoietically-healthy individuals for 72hours. Human MM cell lines were washed thoroughly from the adherent stroma twice with 1xPBS. Stromal cells were lysed in TRIzol and processed by chloroform/isopropanol isolation. RNA underwent DNase treatment with RQ1 DNase prior to cDNA manufactured using SuperScriptIII (Invitrogen). Grem1 expression was assessed by quantitative-PCR using the primers sequences stated previously.

Luciferase Proliferation Assay: Grem1-overexpressing and vector-only OP9 stromal cells were seeded into a 24-well plate at 5x10^4 cells per well and allowed to adhere overnight. The following day 5TGM1.Bmx1 MM PC were seeded onto both stromal cell populations and cultured for 72hours. Following incubation, the entire contents of each well was transferred to a corresponding microfuge tube by vigorously pipetting. Cells were collected by spinning at 2000g for 5mins at 4°C. Cells were washed once with PBS. The cell pellet for each well was lysed in 1x cell lysis buffer (supplier name). 20μl of cell lysate was transferred to a 96-well plate. Immediately prior to reading the plate 100μl of luciferase reaction buffer (5mM MgCl2, 30mM HEPES, 150μM ATP, 250μM of Coenzyme A and 150μg/mL D-luciferin) was added to the cell lysate. Bioluminescence was measured using a Wallac 1420 Victor Microplate reader (Perkin Elmer, Massachusetts, USA), with light intensity used as a direct correlation of MM PC number.

Targeting Grem1 in an immunocompetent murine model of systemic MM: C57BL/KaLwRij mice (6-8-week-old) were inoculated with $5x10^5$ 5TGM1.Bmx1 cells via tail vein injection. Three days post-inoculation with 5TGM1.Bmx1 MM PCs, KaLwRij mice were administered with 30mg/kg Grem1-neutralising antibody Ab7326 or IgG control Ab101.4 (UCB-Pharma, UK) by subcutaneous (s.c.) injection. Mice were treated every 3 days for the duration of the 4-week model. Tumour burden was monitored weekly by BLI as previously described. At the end of the experiment, blood was collected by tail bleed, centrifuged at 2100 g for 10min and serum collected. Serum paraprotein levels were analysed using the Hydragel 30 β1β2 kit (Sebia Electrophoresis, Georgia, USA) following the instructions of the manufacturer. The band corresponding to paraprotein was quantitated relative to serum albumin levels. (SAHMRI Animal Ethics SAM165)

Statistics: Numerical data are presented as mean ± standard error of the mean (S.E.M.). Data representing two testing conditions were analysed by Student's T-Test. Data with more than two testing conditions were analysed by one-way analysis of variance (ANOVA) followed by a Tukey multiple comparison post-hoc test to determine the statistical significance of differences. All statistical analyses were performed using GraphPad Prism 7 (GraphPad Software, Inc, San Diego, CA). All experiments were performed in triplicate.

Description of Results

Grem1 expression is upregulated in MM bone marrow stroma

The expression of Grem1 was analysed in mRNA samples obtained from healthy- and MM patient- derived BM stroma. MM patient BM stroma (n=15) had significantly higher expression of Grem1 compared to BM stroma from age-matched haematopoietically normal donors (n=17) ($p<0.001$) (Fig 10). Grem1 expression was also investigated in the 5TGM1/KaLwRij.Hsd mouse model of myeloma. Compact bone was isolated from healthy- and MM tumour bearing- C57BL/KaLwRij.Hsd mice and analysed for differences in Grem1 expression. BM stroma from tumour-bearing mice demonstrated no significant increase in the expression of Grem1 compared to the healthy controls, however a trend toward increased Grem1 expression was observed (Fig 11A). Importantly, the mice with the greatest tumour burden, as determined by bioluminescent imaging, displayed the

greatest expression of Grem1 (Fig 11B). This result demonstrates that the increase in Grem1 in response to MM tumour growth supports the finding in the human setting.

MM cells promote increased Grem1 expression in BM stroma

Co-culture experiments utilizing murine MM cell line 5TGM1.Bmx1 with the BM-derived stromal cell line, OP9 were performed to determine the effect of MM PCs on Grem1 expression within the BM microenvironment. Following 72 hours of co-culture, expression of Grem1 was significantly greater in the BM stromal cells cultured in the presence of the MM PC than those that did not have contact with the MM PC line ($p<0.05$) (Fig 12a). The earlier time points at 24 and 48 hours of co-culture did not demonstrate a significant change in stromal Grem1 expression (Fig 12a). Co-culture was performed in both Transwell and cell contact conditions, however only the cell contact co-cultures exhibited changes in stromal Grem1 expression (Fig 12b). Primary human bone marrow stromal cells derived from haematopoietically normal individuals was also cultured with the human MM cell lines KMS-11, RPMI.8226, H929 and U266. The KMS-11 and U266 cell lines demonstrated an ability to induce an increase in Grem1 expression from BM stroma after 72 hours of co-culture, while co-culture with cell lines RPMI.8226 and H929 did not result in changes in Grem1 expression in the stroma (Fig 13).

Increased Grem1 expression promotes MM PC proliferation

To further investigate the importance of Grem1 in the setting of MM, stromal cells overexpressing Grem1 were generated and used in co-cultures with 5TGM1 MM PCs. MM PCs demonstrated a significant increase in proliferation when co-cultured with the Gremlin1-overexpressing stromal cells, both in transwell and cell-contact culture settings (Fig 14) ($p<0.01$ and $p<0.001$, respectively). The MM PC co-cultured with the Grem1-overexpressing BM stroma, subsequently exhibited a reduction in BMP signalling pathway activation, as demonstrated by a reduction in the phosphorylation of Smads-1/5/9 (Fig 16). The BMP pathway is known to inhibit MM PC proliferation and promote apoptosis and represents a potential mechanism for the reduction in MM PC proliferation observed (Hjertner *et al.*, 2001; Holien *et al* 2012).

Targeting Grem1 reduces MM tumour burden *in vivo*

To examine the importance of Gremlin1 in MM tumour establishment and growth in vivo we utilized the 5TGM1/KaLwRij mouse MM model. Following disease initiation, mice were randomly assigned (n=13/treatment group) to receive treatment with Gremlin1 neutralising antibody (Ab7326, UCB-CellTech, UK) or IgG control, and disease burden was monitored weekly over four-weeks via bioluminescent imaging. These studies demonstrated that treatment with an anti-Gremlin1 therapy significantly reduced MM tumour burden *in vivo* (Fig 15) (two-way ANOVA, p=0.0056). SPEP analysis of disease burden demonstrated a comparable result, with mice treated with the anti-Grem1 antibody having a significantly lower M-spike intensity relative to serum albumin, compared to the control treated mice.

Discussion

The findings of increased Grem1 in the BM stroma of MM patients are consistent with previous studies that have reported increased Gremlin1 produced by microenvironmental cell populations. In addition, a trend toward increased Grem1 expression in the bones of mice with MM-like tumours was observed. When further studies were conducted utilizing the cell line used for the *in vivo* model, it was shown that culturing the mouse MM PC line directly upon a stromal cell line derived from the BM, that there was a subsequent increase in Grem1 expression from the stroma. This finding was not replicated when the MM PCs where unable to have direct cell-to-cell contact with the stromal cells, however a trend toward increased expression was observed. This indicates that the MM cell-derived factors responsible for the increased Grem1 expression from the stroma rely primarily on cell contact. This was also observed in two of the four human MM cell lines cultured with normal human stroma. The MM cell lines KMS-11 and U266 were able to induce an increase in Grem1 expression, but the RPMI.8226 and H929 cell lines were not. The ability of some human MM cell lines to induce a change in Grem1 expression, but not others may indicate that there are fundamental differences in the expression of key factors responsible for the regulation of Grem1 between these cell lines, with preliminary studies suggesting a role for interleukin-6 (IL-6) in the regulation of Grem1 in MM (Fig 17). Further study is also required to determine whether the ability of the cell line to induce stromal Grem1 expression, correlates with their proliferative response to Grem1.

To examine the role of Grem1 in MM, BM stromal cells overexpressing Grem1 were generated and co-cultured with MM PCs in order to partially replicate the cross-talk between tumour microenvironment and tumour cells. MM PCs demonstrated a significant increase in proliferation in co-culture with Grem1 overexpressing stromal cells. A decrease in the downstream activation of BMP signalling was observed, as indicated by decreased phosphorylation of Smads 1, 5 and 9 in response to increased Grem1 (Fig 16).

As Grem1 displays clear mitogenic roles in MM, we investigated whether therapeutically targeting Grem1 represented a treatment option. Use of the Grem1-neutralising antibody Ab7326 in the 5TGM1/KaLwRij mouse model of MM demonstrated almost a 50% reduction in MM tumour burden. As the 5TGM1 MM PC do not express Grem1, this effect can be attributed a purely a result of targeting the microenvironment.

In summary, this study shows that Grem1 represents a therapeutic target in MM.

Example 14 - Pre-treatment of Grem1 in a mouse model of MM

Introduction

Application of the Grem1-neutralising antibody 3 days after inoculation of mice with multiple myeloma (MM) tumour resulted in a reduction in tumour burden of approximately 50%. This established a clear role for Grem1 in the progression of MM disease, however it remained to be determined if Grem1 played a role in MM disease initiation. To address this question, Grem1-neutralising antibody was administered to the 5TGM1/KaLwRij mouse model prior to tumour inoculation.

Methods

Targeting Grem1 prior to tumour cell inoculation in an immunocompetent murine model of systemic MM: C57BL/KaLwRij mice (6-8-week-old) were administered two doses of 30 mg/kg Grem1-neutralising antibody Ab7326 or IgG control Ab101.4 (UCB-Pharma, UK) by subcutaneous (s.c.) injection three days and one day prior to tumour inoculation. At Day 0, mice were inoculated with $5x10^5$ 5TGM1.Bmx1 cells via tail vein

injection. Mice continued to receive either 30mg/kg Grem1-neutralising antibody Ab7326 or IgG control Ab101.4 (UCB-Pharma, UK) by s.c. injection every 3 days for the duration of the 4-week model. Tumour burden was monitored weekly by BLI as previously described. At the end of the experiment, blood was collected by tail bleed, centrifuged at 2100 g for 10min and serum collected. Serum paraprotein levels were analysed using the Hydragel 30 β1β2 kit (Sebia Electrophoresis, Georgia, USA) according to the manufacturer's instructions. The band corresponding to paraprotein was quantitated relative to serum albumin levels. (SAHMRI Animal Ethics SAM165)

Results/Discussion

Our previous data demonstrated that Grem1 can be targeted to reduce MM tumour burden in the 5TGM1/KaLwRij mouse model of myeloma. This established that Grem1 is a key microenvironmental factor that contributes to disease progression and can be targeted therapeutically. However, it was still unclear whether Grem1 also plays a role in the initiation of MM disease. To address this question, mice received the Grem1-neutralising antibody treatment, or IgG control, prior to MM tumour cell inoculation. As shown in Figure 18, a reduction in tumour burden of approximately 75% at 4-weeks post-tumour cell inoculation was observed.

Blocking Grem1 prior to the administration of MM tumour cells, showed an additional 25% reduction in overall tumour burden by the end of the study, when compared with treatment following tumour cell inoculation. The increased efficacy of the Grem1-neutralising antibody when administered prior to the initiation of tumour model, suggests a role for Grem1 in the initiation of MM disease.

Example 15 – Grem1-induced proliferation of breast cancer cells and antagonism of Grem1 effects with an anti-Grem1 antibody

Materials and Methods

Real-Time Polymerase Chain Reaction (RT-PCR)

Human MDA-MB-231-TXSA breast cancer cells and human MF9 mammary fibroblasts were seeded into 6-well plates and cultured in either normoxic or hypoxic conditions for 48

hrs. Normoxic conditions were maintained at 37°C with 5% CO_2, 20% O_2, while hypoxic conditions were maintained in a hypoxic *in vitro* chamber at 5% O_2, 10% CO_2 (Coy Laboratory Products, Grass Lake, MI, USA). Total RNA was extracted using TRIzol (Life Technologies, Calsbad, CA, USA). After removing genomic DNA contamination with DNase I (Promega, Madison, WI, USA), superscript IV (Invitrogen, Carlsbad, CA) was used to synthesize cDNA. 1 μg of each RNA sample was reverse transcribed and Grem1 gene expression was quantified by RT-PCR using SYBR Green Fluor qPCR mastermix (Qiagen) on a Biorad CFX Connect. Experiments were performed in triplicate with gene expression standardised to β-actin, applying the $2^{-\Delta CT}$ method. Data is presented as fold change relative to normoxia control.

Primer pairs:
human Grem1:
5'-AGGCCCAGCACAATGACTCAG-3' (forward) (SEQ ID NO: 40),
5'-GTCTCGCTTCAGGTATTTGCG-3' (reverse) (SEQ ID NO: 41);
β-actin:
5'-GATCATTGCTCCTCCTGAGC-3' (forward) (SEQ ID NO: 42),
5'–GTCATAGTCCGCCTAGAAGCAT-3' (reverse) (SEQ ID NO: 43).

Proliferation assays in hypoxia

Luciferase expressing MDA-MB-231-TXSA cells were seeded in 96-well microtiter plates ($1x10^4$ cells/well). Cells were cultured either under normoxic or hypoxic conditions and allowed to adhere overnight. Cells were then serum starved for 6 hrs before treatment with increasing concentrations of rhGrem1 protein (0-1000 ng/ml). At 24 and 48 hr time points, luciferase expression was evaluated and used as a surrogate measure of cell number. Briefly, cells were washed in 1xPBS, and subsequently lysed in cell culture lysis buffer (Promega). Luciferase Assay Reagent (5 mM $MgCl_2$, 30 mM HEPES, 150 μM ATP, 50 mg/mL Coenzyme A and 150 μg/mL D-luciferin (Biosynth AG, Staad, Switzerland)) was added to cell lysates, and luminescence was quantified immediately using a luminometer plate reader (Wallac 3000).

Transwell co-culture proliferation assays

Luciferase-expressing murine 4T1 breast cancer cells (1×10^4 cells) were seeded into 3 μm polycarbonate membrane transwells (Costar, Washington, D.C., USA) in a 24-well plate. Following overnight culture, 4T1 cells were starved in serum free DMEM for 6 hrs to synchronise cell growth. GFP+ murine stromal OP9 cells, previously transduced with a control or Grem1 expressing pLEGOiT2 construct were seeded into lower chambers ($2x10^4$ cells) in 10% FCS DMEM. Cells were co-cultured for 72 hrs, before analysing 4T1 cell proliferation via luciferase expression.

Western blot analysis

Murine 4T1 BrCa cells were seeded into 6-well plates and cultured until 80% confluent. Cells were starved in serum-free medium overnight, and then stimulated with indicated treatments for 2 hrs. Cell lysates were prepared and equivalent amounts of protein (50 μg) were separated on 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred to nitrocellulose membranes. Immunoblotting was performed with phospho-Smad1/5/9 antibodies (cell signalling technologies, 1:1000 dilution). Equal loading of protein samples was confirmed by blotting membranes with antibodies against β-actin (Sigma Aldrich, 1:2500 dilution). Proteins were visualised using the Odyssey Infrared Imaging System (LI-COR Bioscience, Lincoln, NE, USA).

Results and Discussion:

We identified that Grem1 induces proliferation of human breast cancer cells under hypoxic conditions. While the mechanism by which Grem1 promotes breast cancer proliferation remains unknown, BMP expression has been shown to be elevated under hypoxia in several other cell types. Therefore, Grem1 may induce proliferation under hypoxia by inhibiting the anti-proliferative effects of BMPs. Human MDA-MB-231-TXSA breast cancer cells and human MF9 mammary fibroblasts were cultured in either normoxic (20% O_2) or hypoxic (5% O_2) conditions for 48 hrs. Quantification of Grem1 mRNA by RT-PCR revealed elevated Grem1 expression under hypoxia in both MDA-MB-231-TXSA cells ($p=0.0024$) and MF9 cells ($p=0.034$) compared to normoxic controls (Fig 19).

When cultured under hypoxic conditions, rhGrem1 stimulated a dose-dependent increase in MDA-MB-231-TXSA proliferation, with a maximal 1.5-fold ($p<0.005$) and 1.7-fold ($p<0.0005$) compared to normoxic conditions, after 24 hr and 48 hr respectively (Fig 20).

To further investigate if Grem1 induced proliferation of breast cancer cells in the context of other stromal factors, murine 4T1 breast cancer cells were co-cultured with stromal OP9 cells expressing either Grem1 or a control vector. Stromal cell-derived Grem1 significantly increased breast cancer cell proliferation by 1.8-fold ($p<0.0005$) after 72hr culture (Fig 21). This further suggests that Grem1 may induce proliferation by antagonising stromal derived BMPs.

As Grem1 is a known BMP-2/4/7 antagonist, we assessed the ability of UCB antibody Ab7326 to inhibit Grem1-induced inhibition of Smad1/5/9 phosphorylation via western blot analysis. Murine 4T1 breast cancer cells exposed to 10 ng/ml BMP2 which led to Smad1/5/9 phosphorylation. Co-incubation with 100 ng/ml rhGrem1 inhibited this BMP2-mediated Smad1/5/9 phosphorylation. Notably, compared to 4T1 cells exposed to rhGrem1 pre-incubated with an isotype control, pre-incubation of rhGrem1 with Ab7326, partially restored Smad1/5/9 phosphorylation in 4T1 cells (Fig 22). These results provide confirmation that Ab7326 can effectively target and neutralise the effects of Grem1.

Example 16 – The effect of monotherapy and combination therapy in VG/Min mice

Mice were treated with 30 mg/kg weekly anti-Grem1 antibody and/or two intraperitoneal daily doses of 40 mg/kg 5-fluorouracil (5FU, Sigma) at or from the age of 35 days onwards, which is very late in the *Vil1-Grem1/Apc^{Min}* disease process. To generate Kaplan-Meier data, mice were sacrificed when humane-end points were reached (mice exhibited anaemia, hunching and inactivity).

Monotherapy with either 5FU or anti-Grem1 in this late stage of disease had no effect on the lifespan of the mice, but combination therapy significantly prolonged mouse lifespan (median survival: no treatment 45 days (n=9), 5FU alone 47 days (n=4), anti-Grem1 alone 49.5 days (n=6), combination therapy 76.5 days (n=6): log rank $p<0.01$), see Figure 23.

These data show the efficacy of combination therapy in late stage, established disease, using anti-Grem1 antibody together with standard chemotherapy targeted against the proliferating epithelium.

Example 17 – The effect of anti-Grem1 antibody in human Patient Derived Xenograft (PDX) models

The efficacy of the anti-gremlin-1 Ab7326 as a mouse IgG1 will be tested in eight Patient Derived Xenograft (PDX) models. The human PDX models were selected based on their increased expression of the GREM1 gene product over control tissue. The models selected include xenografts derived from pancreatic, lung, renal, colorectal, gastric, head and neck and liver cancers. Ab7326 will be dosed weekly subcutaneously. Tumour size will be evaluated throughout the model duration and tumours and plasma samples will be collected and analysed at the end of the experiments.

Example 18 – The effect of anti-Grem1 antibody on tumour burden in C57Bl6/KaLwRij mice (Figure 33)

It was observed that tumour burden in C57Bl6/KaLwRij mice was significantly reduced in the hindlimb bones when mice are treated with Grem1-neutralising antibody, Ab7326 (see Figure 33). A significant reduction in tumour burden was seen when the anti-Grem1 antibody was administered after tumour cell inoculation (Figure 33A), as compared to IgG control (measured by BLI). A significant reduction in tumour burden was also seen when the anti-Grem1 antibody was administered prior to tumour cell inoculation (Figure 33B), again as compared to IgG control (measured by BLI). A downwards trend in splenic tumour burden was also observed in groups treat-ed with anti-Grem1 antibody, in both the (Figure 33C) post-, or (Figure 33D) pre-tumour cell inoculation groups.

C57Bl6/KaLwRij mice are a model for multiple myeloma (MM) in which MM cells grow within the bone marrow (BM) and the spleen. The data presented in Figure 33 demonstrate that anti-Grem1 treatment targets BM-associated tumours as well as MM cells in the spleen. Accordingly, anti-Grem1 therapy may be used to treat both bone-resident tumours,

such as osteosarcoma, as well as disseminated cancers, such as liver, breast and/or prostate cancer.

Example 19 - Late stage treatment of established *Vil1-Grem1* and Apc^{Min} polyposis with anti-Grem1 antibody, slows disease progression and reverts *Vil1-Grem1* mouse intestinal phenotype (Figure 34)

Anti-Grem1 antibody (UCB) or control antibody (UCB) were administered at a dose of 30 mg/kg weekly subcutaneous injections, starting at the age of 4 months for Apc^{Min} (Fig 34B) and 5 months for *Vil1-Grem1* mice (Fig 34A).

Mice were aged to allow development of an intestinal polyposis prior to commencement of antibody treatment (at the age of 4 months for Apc^{Min} and 5 months for *Vil1-Grem1* mice). To generate Kaplan-Meier data, mice were sacrificed when humane-end points were reached (mice exhibited anaemia, hunching and inactivity). In both the *Vil1-Grem1* (A) and the Apc^{Min} (B) models, treatment with anti-Grem1 antibody significantly prolonged the lifespan of the mice. Timed sacrifice showed a reversion of the *Vil1-Grem1* intestinal phenotype over 4 weeks of treatment.

Example 20 - Anti-Grem1 antibody treatment protects *Vil1-Grem1* mice from AOM mutagenesis (Figure 35)

Aberrant epithelial Grem1 expression in *Vil1-Grem1* mice promotes an epithelial stem/progenitor cell phenotype (Davis *et al.*, 2015), and we hypothesised that these cells would be susceptible to somatic mutation. We used azoxymethane (AOM) administration to examine the effect of a mutagenic insult on untreated *Vil1-Grem1* and anti-Grem1 treated *Vil1Grem1* mice. AOM is a well-documented mutagen widely utilised in murine models to recapitulate spontaneous CRC carcinogenesis (Neufert and Neurath, 2007). To generate Kaplan-Meier data, mice were sacrificed when humane-end points were reached (mice exhibited anaemia, hunching and inactivity).

Mice were treated with three subcutaneous doses of 30 mg/kg weekly anti-Grem1 antibody followed by three intraperitoneal doses of 10 mg/kg weekly azoxymethane (AOM, Sigma) or vehicle (saline) prior to a further three weeks of 30 mg/kg weekly anti-Grem1 antibody. Treatment commenced at or from the age of 90 days onwards, which is late enough in the *Vil1-Grem1* disease process to ensure expansion of the progenitor cell population and subsequent formation of ectopic crypt foci.

AOM administration markedly enhanced polyp formation in untreated *Vil1-Grem1* mice. Colonic polyp burden was significantly increased in the AOM treated animals (Figure 35B). This negatively impacted mouse survival (Figure 35A): compare group *VG + no treatment* and group *VG + AOM + no treatment*. The increased tumour burden and reduced survival of the AOM-treated animals was partially abrogated by administration of anti-Grem1 antibody in the period surrounding the mutagenic insult: compare group *VG + AOM + no treatment* with group *VG + AOM + αGrem1*. These data suggest that the normalisation of cell fate determination by the anti-Grem1 antibody in *Vil1-Grem1* mice prevents epithelial stem/progenitor promotion and protects the epithelium from the AOM mutagenic insult.

Example 21 - *Foxl1*, *Wnt5A*, and *Wnt 2B* expression is upregulated in *Vil1-Grem1* stroma and this phenotype is abrogated by anti-Grem1 antibody treatment (Figures 36-38)

To investigate the source of Wnt ligand expression in the villus ectopic crypts in antibody treated and untreated *Vil1-Grem1* mice (n=4) we used *Foxl1 in situ* hybridization (ISH) to identify sub-epithelial telocyte cells as described by Shoshkes-Carmel *et al. (2018)*. *Foxl1* expression levels were quantified using the HALO image analysis software. Villus stromal *Foxl1* expression was significantly increased in untreated *Vil1-Grem1* mice ($p = 0.0037$) (Figure 36 and Figure 38C). Treatment with anti-Grem1 antibody restored *Foxl1* expression levels (p-value WT vs antibody-treated *Vil1-Grem1* mice ($p = 0.4864$), indicating the stromal remodelling and Foxl1 cell recruitment is epithelial Grem1 dependent.

In order to determine which mediators were being produced by Foxl1+ cells, multiplex ISH was used to demonstrate the combination of *Foxl1* and *Wnt5A* expression in villus subepithelial fibroblasts (see Figure 37). The staining marked thin structures with long protrusions which resemble the telocytes described by Shoshkes-Carmel *et al.* (2018).

Epithelial Grem1 expression in the *Vil1-Grem1* model causes activation of villus stromal cell populations that subsequently express Wnt ligands 5A and 2B. Treatment with anti-Grem1 antibody prevented stromal cell activation and abrogated Wnt ligand expression (Figure 38A and 38B) indicating that stromal remodelling is epithelial Grem1 dependent.

Methods

In situ hybridization

Paraffin blocks were sectioned (thickness = 0.4 μm) using DEPC (Sigma) treated H_2O and sections were baked for 2 hours at 60 °C. Tissue was deparaffinised and treated with RNAscope Hydrogen Peroxide (ACD 322335) for 10 min at room temperature (RT). Antigen retrieval was performed for 15 minutes using RNAscope 1X Target Retrieval Reagents (1:10 ACD 322000) at 100 °C. Samples were treated with RNAscope Protease Plus (ACD 322331) for 30 minutes at 40 °C using an HybEZ Hybridization System (ACD). Probes were incubated for 2h at 40 °C. Probes used were as follows: LGR4 (ACD 318321), LGR5 (ACD 312171), FZD5 (ACD 404911), FZD7 (ACD 534101), WNT2B (ACD 405031), WNT5A (ACD 316791), WNT5A-C3 (ACD, 316791-C3) and FOXL1 (ACD 407401). Slides were counter-stained using Haematoxylin (brown) or DAPI (Fluorescent).

ISH quantification

Slides were scanned at 20 x magnification using an Aperio CS2 Digital Pathology Scanner (Leica Biosystems). 100 villi per mouse were annotated using the Aperio ImageScope Pathology Slide Viewing Software (Leica Biosystems). *Foxl1* expression levels were quantified using the HALO image analysis platform (PerkinElmer) and expressed as % of DAB staining per area. Two-way ANOVA and multiple comparisons applying Bonferroni correction were used to compare DAB levels between conditions. Differences were

considered significant at a p value of <0.05. Analysis were performed using the statistical software R.

Example 22 - Anti-Grem1 antibody shows a dose response relationship in *Vil1-Grem1;Apc^{Min}* treated mice (Figure 39)

Anti-Grem1 antibody (UCB) or control antibody (UCB) were administered at variable doses of 15, 30 and 60 mg/kg weekly subcutaneous injections biweekly for 6-weeks, followed weekly thereafter, starting at the age of 21 days in *Vil1-Grem1;Apc^{Min}* mice. To generate Kaplan-Meier data (presented in Figure 39), mice were sacrificed when humane-end points were reached (mice exhibited anaemia, hunching and inactivity). Treatment with anti-Grem1 prolonged mouse lifespan in a dose dependent fashion with no additional survival benefit seen from a dose >30 mg/kg weekly.

Example 23 - Comparison of early and late phase treatment with anti-Grem1 antibody in *Vil1-Grem1* mice and *Apc^{Min}* mice (Figure 40)

Anti-Grem1 antibody (UCB) or control antibody (UCB) was administered to *Vil1-Grem1* mice (Figure 40A) and *Apc^{Min}* mice (Figure 40B) at a dose of 30 mg/kg weekly subcutaneous injections, starting at the age of 42 days (early stage treatment) or 120 days after development of an intestinal polyposis (late stage treatment).

To generate Kaplan-Meier data, mice were sacrificed when humane-end points were reached (mice exhibited anaemia, hunching and inactivity). Treatment with the anti-Grem1 antibody initiated at both time points significantly prolonged the lifespan of the mice. Specifically, for the *Vil1-Grem1* mice (Figure 40A) the median survival of the vehicle group (242 days (n=13)) was significantly increased by treatment with anti-Grem1 antibody: late-stage treatment: 519 days; early stage treatment: 540 days. In the *Apc^{Min}* mice (Figure 40B), the median survival of the vehicle treatment group was significantly extended from 192 days to 261 days (late stage treatment) and 424.5 days (early stage treatment) as a result of treatment with anti-Grem1 antibody.

Example 24 – use of a Grem1-neutralising antibody significantly reduces breast cancer tumour growth within the bone and lung in pre-clinical models of breast cancer (Figures 41-45)

Using novel anti-Grem1 neutralising monoclonal antibodies, the *in vivo* contribution of Grem1 in the establishment and progression of breast cancer was investigated using two clinically relevant pre-clinical mouse models of breast cancer.

Methods

Cell Culture

The PyMT-B01 mouse-derived breast cancer cell was kindly donated by Professor Sheila Stewart (St Louis, MO, USA) and the MDA-MB-231 human breast cancer cell line was kindly provided by Dr Toshiyuki Yoneda (formerly at University of Texas Health Sciences Centre, San Antonio, TX). Both cell lines express luciferase produced by retroviral expression of the SFG-NES-TGL vector (Ponomarev, V., et al. Eur J Nucl Med Mol Imaging, 2004. 31(5): p. 740-51. Cancer cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Life Technologies, Australia) supplemented with 10 % foetal bovine serum (FBS, Life Technologies, Australia), 100 IU/mL penicillin (Life Technologies, Australia), 100 µg/mL streptomycin (Life Technologies, Australia) and 25 mM HEPES (Life Technologies, Australia) at 37°C in a 5 % CO2 humidified atmosphere.

Animals

Animal studies were performed in accordance with animal protocol procedures approved by the Animal Ethics Committee of the South Australian Health and Medical Research Institute (SAHMRI) under ethics number SAM373, and conform to the guidelines established by the 'Australian Code of Practice for the Care and Use of Animals for Scientific Purposes'.

Grem1 antibody treatment in pre-clinical breast cancer models

PyMT-B01: 5-week old C57BL6 immunocompetent mice were subcutaneously (s.c) administered two 30 mg/kg doses of anti-Grem1 antibody Ab7326 or IgG control,

Ab101.4, the week prior to tumour cell inoculation. 1 x 10^5 luciferase expressing PyMT-B01 mouse breast cancer cells were injected systemically *via* the caudal artery (CA).

MDA-MB-231: 5-week old NOD/SCiD/gamma (NSG) immunocompromised mice were subcutaneously (s.c) administered two 30 mg/kg doses of anti-Grem1 antibody UCB6114 or IgG control, AbA33, the week prior to tumour cell inoculation. 1 x 10^5 luciferase expressing MDA-MB-231 human breast cancer cells were injected systemically *via* the CA.

Treatment continued twice weekly for the duration of the study. At weekly intervals, mice were administered 150 mg/kg luciferin intraperitoneally (i.p) and imaged using the Xenogen IVIS Bioluminescence Imaging System, and tumour burden was quantitated using Living Image software. The study ended on day 13 and day 22 post-tumour cell injection for the PyMT-B01 and MDA-MB-231 models respectively, according to animal ethics requirements. Organs were dissected upon culling animals and BLI imaged *ex vivo* for analysis of tumour metastasis.

Results

At the conclusion of the study, the PyMT-B01 tumour bearing mice treated with Grem1-neutralising antibody, Ab7326, had a 35 % reduction in overall tumour burden, compared to mice treated with isotype control, Ab101.4, when tumour was injected *via* the CA (Figure 41). This BLI signal is primarily concentrated in the hindlimbs, indicative of the extensive skeletal tumour involvement associated with this model. A statistically significant reduction in lung metastatic tumour burden was also observed in mice treated with Grem1-neutralising antibody compared to isotype controls (Figure 42A). A downward trend in liver metastatic tumour burden was also observed in the PyMT-B01 tumour-bearing mice treated with the Grem1-neutralising antibody (Figure 42B).

At the conclusion of the second study (day 22), the mean tumour burden for MDA-MB-231 tumour-bearing NSG mice was lower in the Grem1-neutralising antibody (UCB6114) treatment group compared to the control group treated with AbA33 (Figure 43).

A reduction in mean tumour burden for lung metastasis in mice treated with the anti-Grem1 antibody (UCB6114) was also observed (Figure 45).These results show that targeting Grem1 with a Grem1-neutralising antibody significantly reduces breast cancer tumour growth within the bone and lung. These studies support the hypothesis that anti-Grem1 therapy may represent a suitable treatment option in metastatic breast cancer. In conclusion, the present inventors provide the first evidence that targeting Grem1 with a Grem1-neutralising antibody *in vivo* represents an effective therapeutic strategy for reducing breast cancer skeletal and lung metastatic tumour growth.

Example 25 – use of a Grem1-neutralising antibody reduces prostate cancer tumour growth in a pre-clinical model of prostate cancer (Figures 46 and 47)

Using the novel anti-Grem1 neutralising monoclonal antibody (UCB6114), the *in vivo* contribution of Grem1 in the establishment and progression of prostate cancer in a pre-clinical murine models of prostate cancer was investigated.

Methods

Cell Culture

The PC-3 human prostate cancer cell line was obtained from ATCC (Manassas, VA, USA). The cell line expresses luciferase produced by retroviral expression of the SFG-NES-TGL vector (Ponomarev, V., et al. 2004). Cancer cells were cultured in RPMI1640 (Life Technologies, Australia) supplemented with 10 % foetal bovine serum (FBS, Life Technologies, Australia), 100 IU/mL penicillin (Life Technologies, Australia), 100 µg/mL streptomycin (Life Technologies, Australia) and 25 mM HEPES (Life Technologies, Australia) at 37 °C in a 5 % CO_2 humidified atmosphere.

Animals

Animal studies were performed in accordance with animal protocol procedures approved by the Animal Ethics Committee of the South Australian Health and Medical Research Institute (SAHMRI) under ethics number SAM373, and conform to the guidelines

established by the 'Australian Code of Practice for the Care and Use of Animals for Scientific Purposes'.

Grem1 antibody treatment in pre-clinical prostate cancer models

5-week old NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG) immunocompromised mice were subcutaneously (s.c) administered two 30 mg/kg doses of anti-Grem1 antibody (UCB6114) or IgG control, AbA33, the week prior to tumour cell inoculation. 5 x 10^5 luciferase expressing PC-3 mouse prostate cancer cells were injected systemically *via* the caudal artery (CA).

Antibody treatment continued twice weekly for the duration of the study. A t weekly intervals, mice were administered 150 mg/kg luciferin intraperitoneally (i.p) and imaged using the Xenogen IVIS Bioluminescence Imaging System, and tumour burden was quantitated using Living Image software (PerkinElmer, MA, USA). At the end of each study, organs were dissected upon culling animals and BLI imaged *ex vivo* for analysis of tumour metastasis.

Results

The PC-3 prostate cancer model showed significant liver tumour burden (another common site of prostate cancer metastasis) from day 7, in addition to evidence of skeletal tumour burden by BLI. A significant reduction in whole animal (Figure 46A) and liver (Figure 46B) tumour burden was observed in mice treated with Grem1-neutralising antibody (UCB6114) as compared to mice treated with isotype control antibody (AbA33). A downward trend in hindlimb (Figure 46C) and lung (Figure 46D) tumour burden was also observed for the mice treated with Grem1-neutralising antibody.

SEQUENCE LISTING

SEQ ID NO: 1 (Human Gremlin-1; Uniprot ID: O60565)

MSRTAYTVGALLLLLGTLLPAAEGKKKGSQGAIPPPDKAQHNDSEQTQSPQQPGSRNRGRGQGRGTAMPGEEV
LESSQEALHVTERKYLKRDWCKTQPLKQTIHEEGCNSRTIINRFCYGQCNSFYIPRHIRKEEGSFQSCSFCKP
KKFTTMMVTLNCPELQPPTKKKRVTRVKQCRCISIDLD

SEQ ID NO: 2 (Human truncated Gremlin-1 used in crystallography with N-terminal tag)

MGSSHHHHHHSSGENLYFQGSAMPGEEVLESSQEALHVTERKYLKRDWCKTQPLKQTIHEEGCNSRTIINRFC
YGQCNSFYIPRHIRKEEGSFQSCSFCKPKKFTTMMVTLNCPELQPPTKKKRVTRVKQCRCISIDLD

SEQ ID NO: 3 (Ab7326 HCDR1 combined Kabat & Chothia)
GYTFTDYYMH

SEQ ID NO: 4 (Ab7326 HCDR1 Kabat)
DYYMH

SEQ ID NO: 5 (Ab7326 HCDR2 Kabat)
LVDPEDGETIYAEKFQG

SEQ ID NO: 6 (Ab7326 HCDR3 Kabat)
DARGSGSYYPNHFDY

SEQ ID NO: 7 (Ab7326 LCDR1 Kabat)
KSSQSVLYSSNNKNYLA

SEQ ID NO: 8 (Ab7326 LCDR2 Kabat)
WASTRES

SEQ ID NO: 9 (Ab7326 LCDR3 Kabat)
QQYYDTPT

SEQ ID NO: 10 (Ab7326 Heavy chain variable region variant 1)
QVQLVESGAEVKKPGATVKISCKVSGYTFTDYYMHWVQQAPGKGLEWMGLVDPEDGETIYAEKFQGRVTITAD
TSTDTAYMELSSLRSEDTAVYYCATDARGSGSYYPNHFDYWGQGTLVTVSS

SEQ ID NO: 11 (Ab7326 Light chain variable region variant 1)
DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS
GTDFTLTINSLQAEDVAVYFCQQYYDTPTFGQGTRLEIK

SEQ ID NO: 12 (Ab7326 Heavy chain variable region variant 2)
QVQLVQSGAEVKKPGATVKISCKVSGYTFTDYYMHWVQQAPGKGLEWMGLVDPEDGETIYAEKFQGRVTITAD
TSTDTAYMELSSLRSEDTAVYYCATDARGSGSYYPNHFDYWGQGTLVTVSS

SEQ ID NO: 13 (Ab7326 Light chain variable region variant 2)
DIVMTQTPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS
GTDFTLTINSLQAEDVAVYFCQQYYDTPTFGQGTRLEIK

SEQ ID NO: 14 (Mouse full length IgG1 heavy chain variant 1)
QVQLVESGAEVKKPGATVKISCKVSGYTFTDYYMHWVQQAPGKGLEWMGLVDPEDGETIYAEKFQGRVTITAD
TSTDTAYMELSSLRSEDTAVYYCATDARGSGSYYPNHFDYWGQGTLVTVSSAKTTPPSVYPLAPGSAAQTNSM
VTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDK
KIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQFSWFVDDVEVHTAQTQP
REEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVS
LTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHT
EKSLSHSPGK

SEQ ID NO: 15 (Mouse full length IgG1 light chain variant 1)
DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS
GTDFTLTINSLQAEDVAVYFCQQYYDTPTFGQGTRLEIKRTDAAPTVSIFPPSSEQLTSGGASVVCFLNNFYP
KDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

SEQ ID NO: 16 (Human full length IgG1 heavy chain variant 2)
QVQLVQSGAEVKKPGATVKISCKVSGYTFTDYYMHWVQQAPGKGLEWMGLVDPEDGETIYAEKFQGRVTITAD
TSTDTAYMELSSLRSEDTAVYYCATDARGSGSYYPNHFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD

KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGK

SEQ ID NO: 17 (Human full length IgG1 light chain variant 2)

DIVMTQTPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS
GTDFTLTINSLQAEDVAVYFCQQYYDTPTFGQGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 18 (Fab heavy chain variant 1)

QVQLVESGAEVKKPGATVKISCKVSGYTFTDYYMHWVQQAPGKGLEWMGLVDPEDGETIYAEKFQGRVTITAD
TSTDTAYMELSSLRSEDTAVYYCATDARGSGSYYPNHFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KKVEPKSC

SEQ ID NO: 19 (Fab light chain variant 1)

DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS
GTDFTLTINSLQAEDVAVYFCQQYYDTPTFGQGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 20 (Human truncated Gremlin-1 used in crystallography without N-terminal tag)

AMPGEEVLESSQEALHVTERKYLKRDWCKTQPLKQTIHEEGCNSRTIINRFCYGQCNSFYIPRHIRKEEGSFQ
SCSFCKPKKFTTMMVTLNCPELQPPTKKKRVTRVKQCRCISIDLD

SEQ ID NO: 21 (Mature Gremlin-1 sequence of SEQ ID NO: 1 lacking the signal peptide of amino acids 1-21)

KKKGSQGAIPPPDKAQHNDSEQTQSPQQPGSRNRGRGQGRGTAMPGEEVLESSQEALHVTERKYLKRDWCKTQ
PLKQTIHEEGCNSRTIINRFCYGQCNSFYIPRHIRKEEGSFQSCSFCKPKKFTTMMVTLNCPELQPPTKKKRV
TRVKQCRCISIDLD

SEQ ID NO: 22 (Human IgG4P heavy chain variant 1)

QVQLVESGAEVKKPGATVKISCKVSGYTFTDYYMHWVQQAPGKGLEWMGLVDPEDGETIYAEKFQGRVTITAD
TSTDTAYMELSSLRSEDTAVYYCATDARGSGSYYPNHFDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSEST
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVD
KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHN
HYTQKSLSLSLGK

SEQ ID NO: 23 (Human IgG4P light chain variant 1)

DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS
GTDFTLTINSLQAEDVAVYFCQQYYDTPTFGQGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 24 (Human IgG1 heavy chain DNA variant 1)

caagtgcaactggtggaatccggggccgaagtgaaaaagcccggagccactgtgaagatctcttgcaaagtgt
ccggctacaccttcaccgactattacatgcactgggtccagcaggcacctgggaagggccttgagtggatggg
tctggtcgatcccgaggacggcgaaactatctacgccgagaagttccagggtcgcgtcaccatcaccgccgac
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gcggctctcggttgcctcgtgaaggactacttcccggagccggtgaccgtgtcgtggaacagcggagccctga
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SEQ ID NO: 25 (Human IgG1 light chain DNA variant 1)

gacattgtgatgacccagtcccccgattcgcttgcggtgtccctgggagaacgggccaccattaactgcaaga
gctcacagtccgtcctgtattcatcgaacaacaagaattacctcgcatggtatcagcagaagcctggacagcc
tcccaagctgctcatctactgggctagcacccgcgaatccggggtgccggatagattctccggatcgggttcg
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ggtgtacgcctgcgaagtgacccaccagggcctgtccagccccgtgaccaagtccttcaaccggggcgagtgc

SEQ ID NO: 26 (Human IgG4P heavy chain DNA variant 1)

caagtgcaactggtggaatccggggccgaagtgaaaaagcccggagccactgtgaagatctcttgcaaagtgt
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cactacacccagaagtccctgtccctgagcctgggcaag

SEQ ID NO: 27 (Human IgG4P light chain DNA variant 1)

gacattgtgatgacccagtcccccgattcgcttgcggtgtccctgggagaacgggccaccattaactgcaaga
gctcacagtccgtcctgtattcatcgaacaacaagaattacctcgcatggtatcagcagaagcctggacagcc
tcccaagctgctcatctactgggctagcacccgcgaatccggggtgccggatagattctccggatcgggttcg
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ggtgtacgcctgcgaagtgacccaccagggcctgtccagccccgtgaccaagtccttcaaccggggcgagtgc

SEQ ID NO: 28 (Mouse full length IgG1 heavy chain variant 2)

QVQLVQSGAEVKKPGATVKISCKVSGYTFTDYYMHWVQQAPGKGLEWMGLVDPEDGETIYAEKFQGRVTITAD
TSTDTAYMELSSLRSEDTAVYYCATDARGSGSYYPNHFDYWGQGTLVTVSSAKTTPPSVYPLAPGSAAQTNSM
VTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDK
KIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQFSWFVDDVEVHTAQTQP
REEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVS
LTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHT

EKSLSHSPGK

SEQ ID NO: 29 (Mouse full length IgG1 light chain variant 2)
DIVMTQTPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS
GTDFTLTINSLQAEDVAVYFCQQYYDTPTFGQGTRLEIKRTDAAPTVSIFPPSSEQLTSGGASVVCFLNNFYP
KDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

SEQ ID NO: 30 (Human full length IgG1 heavy chain variant 1)
QVQLVESGAEVKKPGATVKISCKVSGYTFTDYYMHWVQQAPGKGLEWMGLVDPEDGETIYAEKFQGRVTITAD
TSTDTAYMELSSLRSEDTAVYYCATDARGSGSYYPNHFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGK

SEQ ID NO: 31 (Human full length IgG1 light chain variant 1)
DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS
GTDFTLTINSLQAEDVAVYFCQQYYDTPTFGQGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 32 (Fab heavy chain variant 2)
QVQLVQSGAEVKKPGATVKISCKVSGYTFTDYYMHWVQQAPGKGLEWMGLVDPEDGETIYAEKFQGRVTITAD
TSTDTAYMELSSLRSEDTAVYYCATDARGSGSYYPNHFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KKVEPKSC

SEQ ID NO: 33 (Fab light chain variant 2)
DIVMTQTPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS
GTDFTLTINSLQAEDVAVYFCQQYYDTPTFGQGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 34 (Human IgG4P heavy chain variant 2)
QVQLVQSGAEVKKPGATVKISCKVSGYTFTDYYMHWVQQAPGKGLEWMGLVDPEDGETIYAEKFQGRVTITAD
TSTDTAYMELSSLRSEDTAVYYCATDARGSGSYYPNHFDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSEST
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVD
KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHN
HYTQKSLSLSLGK

SEQ ID NO: 35 (Human IgG4P light chain variant 2)
DIVMTQTPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS
GTDFTLTINSLQAEDVAVYFCQQYYDTPTFGQGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 36 (Human Gremlin-1; full sequence)
actcggtgcgccttccgcggaccgggcgacccagtgcacggccgccgcgtcactctcggtcccgctgaccccg
cgccgagccccggcggctctggccgcggccgcactcagcgccacgcgtcgaaagcgcaggccccgaggacccg
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CLAIMS

1. An anti-GREM1 antagonist for use in a method for the treatment or prevention of a cancer.

2. An anti-GREM1 antagonist for use according to claim 1, wherein the cancer is a solid cancer.

3. An anti-GREM1 antagonist for use according to claim 1 or 2, wherein the cancer has stromal GREM1 overexpression.

4. An anti-GREM1 antagonist for use according to any preceding claim, wherein the cancer is selected from colorectal cancer, multiple myeloma, pancreatic cancer, bladder cancer, breast cancer, lung cancer, stomach cancer, duodenal cancer, oesophageal cancer, head and neck cancer, prostate cancer, glioma, endometrial cancer, liver cancer, spleen cancer, bone-resident cancer, and osteosarcoma.

5. An anti-GREM1 antagonist for use according to claim 4, wherein the cancer is colorectal cancer.

6. An anti-GREM1 antagonist for use according to claim 5, wherein the colorectal cancer is a mesenchymal subtype colorectal cancer.

7. An anti-GREM1 antagonist for use according to claim 4, wherein the cancer is multiple myeloma.

8. An anti-GREM1 antagonist for use according to claim 4, wherein the cancer is breast cancer.

9. An anti-GREM1 antagonist for use according to any one of the preceding claims, wherein the cancer has epithelial GREM1 overexpression.

10. An anti-GREM1 antagonist for use according to claim 9, wherein the cancer is a GREM1-initiated cancer.

11. An anti-GREM1 antagonist for use according to any one of the preceding claims, wherein the cancer is a disseminated cancer.

12. An anti-GREM1 antagonist for use according to any one of the preceding claims, wherein the cancer is an established cancer, optionally wherein the established cancer is an established colorectal cancer.

13. An anti-GREM1 antagonist for use according to any one of the preceding claims, wherein the antagonist is a peptide, a protein, an antibody, a polynucleotide, an oligonucleotide, an antisense RNA, a small interfering RNA (siRNA), a small molecule inhibitor or a small hairpin RNA (shRNA).

14. An anti-GREM1 antagonist for use according to claim 13, wherein the antagonist is an antibody which binds to an epitope on Gremlin-1 comprising at least one residue selected from Ile131, Lys147, Lys148, Phe149, Thr150, Thr151, Arg169, Lys174 and Gln175, wherein the residue numbering is according to SEQ ID NO: 1.

15. An anti-GREM1 antagonist for use according to claim 14, wherein the antibody binds an epitope comprising all of Ile131, Lys147, Lys148, Phe149, Thr150, Thr151, Arg169, Lys174 and Gln175.

16. An anti-GREM1 antagonist for use according to claim 14 or 15, wherein Lys147, Lys148, Phe149, Thr150, Thr151, Arg169, Lys174 and Gln175 are located on the same Gremlin-1 monomer and Ile131 is located on the second Gremlin-1 monomer.

17. An anti-GREM1 antagonist for use according to claim 13, wherein the antagonist is an anti-Gremlin-1 antibody which comprises heavy chain complementarity determining region (HCDR) sequences contained within a heavy chain variable region (HCVR) of SEQ

ID NO: 10 or 12 and/or light chain complementarity determining region (LCDR) sequences contained within a light chain variable region (LCVR) of SEQ ID NO: 11 or 13.

18. An anti-GREM1 antagonist for use according to claim 13, wherein the antagonist is an anti-Gremlin-1 antibody which comprises at least one HCDR sequence selected from SEQ ID NOs: 3, 4, 5 and 6 and/or at least one LCDR sequence selected from SEQ ID NOs: 7, 8 and 9.

19. An anti-GREM1 antagonist for use according to claim 18, wherein the anti-Gremlin-1 antibody comprises a HCDR3 sequence of SEQ ID NO: 6.

20. An anti-GREM1 antagonist for use according to claim 18 or 19, wherein the anti-Gremlin-1 antibody comprises an HCDR1/HCDR2/HCDR3 sequence combination selected from SEQ ID NOs: 4/5/6 or from SEQ ID NOs:3/5/6 and/or an LCDR1/LCDR2/LCDR3 sequence combination selected from SEQ ID NOs: 7/8/9.

21. An anti-GREM1 antagonist for use according to any one of claims 18 to 20, wherein the anti-Gremlin-1 antibody comprises a HCDR1/HCDR2/HCDR3/LCDR1/LCDR2/LCDR3 sequence combination of SEQ ID NOs: 4/5/6/7/8/9 or SEQ ID NOs: 3/5/6/7/8/9.

22. An anti-GREM1 antagonist for use according to any one of claims 18 to 21, wherein the anti-Gremlin-1 antibody comprises a heavy chain variable region (HCVR) sequence of SEQ ID NO: 10 or 12 and/or a light chain variable region (LCVR) sequence of SEQ ID NO: 11 or 13, or sequences which are at least 95% identical thereto.

23. An anti-GREM1 antagonist for use according to claim 22, wherein the anti-Gremlin-1 antibody comprises a HCVR and LCVR sequence pair of SEQ ID NOs: 10/11 or 12/13 or sequences which are at least 95% identical thereto.

24. An anti-GREM1 antagonist for use according to claim 23, wherein the anti-Gremlin-1 antibody comprises HCDR1/HCDR2/HCDR3/LCDR1/LCDR2/LCDR3 sequences

consisting of SEQ ID NOs: 4/5/6/7/8/9 or SEQ ID NOs: 3/5/6/7/8/9 and the remainder of the HCVR and LCVR comprise at least 95% identity to SEQ ID NOs: 10, 11, 12 and/or 13 respectively.

25. An anti-GREM1 antagonist for use according to claims 22-24, wherein the anti-Gremlin-1 antibody comprises a heavy chain of SEQ ID NO: 14, 16, 18, 22, 28, 30, 32 or 34 and/or a light chain of SEQ ID NO: 15, 17, 19, 23, 29, 31, 33 or 35, or sequences which are at least 95% identical thereto.

26. The anti-GREM1 antagonist for use according to claim 25, wherein the anti-Gremlin-1 antibody comprises a heavy and light chain pair of SEQ ID NOs: 14/15, 16/17, 18/19, 22/23, 28/29 or 30/31, 32/33, 34/35, or sequences which are at least 95% identical thereto.

27. The anti-GREM1 antagonist for use according to claim 26, wherein the HCDR1/HCDR2/HCDR3/LCDR1/LCDR2/LCDR3 sequences of the antibody consist of SEQ ID NOs: 4/5/6/7/8/9 or SEQ ID NOs: 3/5/6/7/8/9 and the remainder of the heavy and light chains comprise at least 95% identity to SEQ ID NOs: 14, 15, 16 and/or 17 respectively.

28. The anti-GREM1 antagonist for use according to claim 13, wherein the antagonist is an antibody which competes for binding to Gremlin-1 with an antibody as defined in any one of claims 15-25.

29. The anti-GREM1 antagonist for use according to claim 13, wherein the antagonist is an antibody which binds the same epitope on Gremlin-1 as an antibody defined in any one of claims 15-25.

30. The anti-GREM1 antagonist for use according to any one of claims 13-29, wherein the antagonist antibody is a chimeric, human or humanised antibody.

31. The anti-GREM1 antagonist for use according to any one of claims 13-30, wherein the antagonist antibody is a Fab, modified Fab, Fab', modified Fab', $F(ab')_2$, Fv, single domain antibody or an scFv.

32. The anti-GREM1 antagonist for use according to claim 13, wherein the antagonist is a polynucleotide encoding an antibody as defined in any one of claims 14-31, or an expression vector carrying said polynucleotide.

33. The anti-GREM1 antagonist for use according to any one of claims 13-31, wherein the antagonist antibody is comprised in pharmaceutical composition further comprising a pharmaceutically acceptable adjuvant and/or carrier.

34. An anti-GREM1 antagonist for use according to any one of the preceding claims, wherein the method comprises separate, sequential or simultaneous administration of an additional anti-cancer agent.

35. An anti-GREM1 antagonist for use according to claim 34, wherein said additional anti-cancer agent is a chemotherapeutic agent.

36. An anti-GREM1 antagonist for use according to any one of the preceding claims, wherein the method comprises separate, sequential or simultaneous radiotherapy.

37. An anti-cancer agent for use in a method for the treatment of a cancer wherein the method comprises separate, sequential or simultaneous administration of an anti-GREM1 antagonist.

38. An anti-cancer agent for use according to claim 37, wherein said anti-cancer agent is a chemotherapeutic agent.

39. An anti-cancer agent for use according to claim 37 or 38, wherein said cancer, said antagonist and/or said method are as defined in any one of claims 1-34.

40. A method of treating a cancer comprising administering a therapeutically effective amount of an anti-GREM1 antagonist to a subject in need thereof.

41. A method according to claim 40, wherein said cancer, said antagonist and/or said method are as defined in any one of claims 1-35.

42. A composition or kit comprising an anti-GREM1 antagonist and an additional anti-cancer agent.

43. A composition or kit according to claim 42, wherein the anti-GREM1 antagonist is as defined in any one of claims 13-32.

44. A composition or kit according to claim 42 or 43, wherein the additional anti-cancer agent is a chemotherapeutic agent.

45. The composition or kit of any one of claims 42 to 44, wherein said anti-cancer or chemotherapeutic agent is suitable for treatment of colorectal cancer, optionally selected from 5-fluoruracil, oxaliplatin, irinotecan, folinic acid, cetuximab, nivolumab or bevacizumab.

46. A composition or kit of any one of claims 42 to 44, wherein said anticancer or chemotherapeutic agent is suitable for treatment of multiple myeloma, optionally selected from an anti-CD38 antibody (such as daratumumab), an anti-SLAMF7 antibody (such as elotuzumab), an anti-IL-6 antibody (such as siltuximab), or bortezumib or iMID (lenalidomide/pomalenomide) or an analogue of either thereof.

47. A method for detecting cancer in a patient, the method comprising measuring stromal expression of GREM1 in the patient, wherein stromal overexpression of GREM1 indicates that the patient comprises a cancer.

48. A method for prognosing a cancer in a patient, the method comprising determining

whether or not the cancer comprises stromal overexpression of GREM1, wherein stromal overexpression of GREM1 in the cancer indicates that the patient has a worse prognosis than in the situation of normal stromal expression of GREM1.

49. A method for determining whether or not a patient having or suspected of having or being at risk of developing cancer is likely to respond to treatment with a chemotherapeutic agent, which method comprises measuring stromal expression of GREM1 in the patient, and thereby predicting whether or not the patient is likely to respond to treatment with the chemotherapeutic agent.

50. A method for determining whether or not a patient having or suspected of having or being at risk of developing cancer is likely to respond to treatment with a GREM1 antagonist, the method comprising measuring stromal expression of GREM1 in the patient, and thereby predicting whether or not the patient is likely to respond to treatment with the GREM1 antagonist.