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DESCRIPTION

FIELD OF THE INVENTION

[0001] The invention relates to medical uses and methods for targeting cancer stem cells, particularly in the treatment of cancer. The present invention also relates to medical uses and methods for the treatment of relapsed or refractory cancer in human patients. The disclosure provides methods of selecting patients who will benefit from treatment of cancer through the medical uses or methods of treatment of the invention.

INTRODUCTION

[0002] The putative existence of a cancer stem cell has been suggested in many human cancers including leukaemias and solid tumours. The cancer stem cell hypothesis considers that only a small sub-population of tumour cells is responsible for the formation and maintenance of the bulk of the tumour. The emergence of this concept can be traced back to the work of Lapidot *et al.* (1994), who showed evidence that only a small percentage of acute myeloid leukaemia cells had the capability to initiate leukaemia in mice. These cells were shown to express similar cell surface markers (CD34⁺/CD38⁻) to normal haematopoietic stem cells implying that a similar hierarchical organisation may occur in tumours. Subsequently, cancer stem cells have been identified in a wide range of solid tumours including breast, lung, colon, prostate, ovarian, skin, and pancreas.

[0003] Conventional anti-cancer approaches are directed predominantly at bulk tumour populations. Such strategies often have limited efficacy because of intrinsic or acquired drug resistance and/or resistance to ionizing radiation, so relapse and the emergence of drug resistance are common features of many cancers. Mechanisms of therapeutic resistance include increased recognition and repair of therapy-induced DNA damage, altered cell cycle checkpoint control, impaired functioning of apoptotic pathways, and reduced drug accumulation as a result of increased expression of ABC transporters that efflux drugs. Evidence has emerged that cancer stem cells have increased resistance to chemo- and radiotherapy and are often enriched for in patients who relapse. Overt cancer stem cell chemo-resistance has been reported in human leukaemias, in malignant melanoma, and in several solid tumours including breast, pancreatic, and colorectal cancers.

[0004] Cancer stem cell-specific phenotypes and functions have been shown to contribute to tumourigenicity, cancer progression, and therapeutic resistance. The persistence of cancer stem cells may also contribute to treatment failure. Therefore, cancer stem cells represent novel and translationally relevant targets for cancer therapeutics.

[0005] NUC-1031, also known as gemcitabine-[phenyl-benzoxy-L-alaninyl]-phosphate or by

the trade name "Acelarin" is a ProTide derivative of the chemotherapeutic agent gemcitabine. Acelarin has been disclosed for use in the treatment of relapsed/refractory cancers (Ghazaly et al., "ProGem1: A phase I/II study of a first-in-class nucleotide analogue Acelarin (NUC-1031 in patients with advanced solid tumours"); and in the treatment of different cancers and cancer cells (McGuigan, "A phosphoramidate ProTide (NUC-1031) and acquired and intrinsic resistance to gemcitabine" J. Clin. Oncol., vol. 29, 2011; Ghazaly et al., "Acelarin: A novel nucleotide analogue that overcomes the key cancer resistance mechanisms with poor survival"; and Slusarczyk, "Application of ProTide Technology to Gemcitabine: A Successful Approach to Overcome the Key Cancer Resistance Mechanisms Leads to a New Agent (NUC-1031) in Clinical Development" J. Med. Chem., vol. 57, 2014).

SUMMARY OF THE INVENTION

[0006] In a first aspect the present invention provides NUC-1031 for use in targeting cancer stem cells in the treatment of cancer.

[0007] In a second aspect the invention provides a method of determining whether a patient with cancer or a pre-cancerous condition will benefit from prevention or treatment of cancer with NUC-1031, the method comprising:

assaying a biological sample representative of cancer or a pre-cancerous condition in the patient for the presence of CSCs; wherein the presence of CSCs in the biological sample indicates that the patient will benefit from treatment with NUC-1031.

[0008] The invention is based upon the finding that NUC-1031 is able to preferentially reduce cancer stem cell (CSC) numbers. This finding is surprising in that CSCs are known to be resistant to many chemotherapeutic agents, and there has previously been no suggestion that either NUC-1031 or gemcitabine, the parent prodrug compound from which NUC-1031 is derived, were able to target CSCs. Thus the finding that NUC-1031 is able to target CSCs and thus reduce their numbers, a finding which the inventors have confirmed is applicable across a broad range of cancers, represents a surprising breakthrough that enables a range of new therapeutic applications of NUC-1031.

[0009] The inventors have surprisingly found that NUC-1031 is able to effectively treat relapsed or refractory cancers in human patients. Studies undertaken by the inventors have shown that administration of NUC-1031 to patients with many different forms of relapsed or refractory cancer is able to provide effective treatment of the cancer. In particular, such administration is able to bring about a reduction in tumour size and/or a reduction in clinically relevant biomarkers that are associated with more favourable prognosis. Furthermore, treatment with NUC-1031 is able to maintain a reduction in the size of tumours in patients with relapsed or refractory cancer. Accordingly, NUC-1031 is able to achieve a high, durable

Disease Control Rate (DCR) in patients with relapsed or refractory cancers.

[0010] Indeed, without wishing to be bound by any hypothesis, the inventors believe that the ability of NUC-1031 to target CSCs contributes to the therapeutic utility of NUC-1031 in the treatment of relapsed or refractory cancer.

[0011] The ability of NUC-1031 to target CSCs provides new therapies directed against those cancer cells that are considered most difficult to treat, and that are considered to play a major role in the resistance that limits effectiveness of many existing cancer therapies. This ability also provides a way of targeting cells that are believed to be associated with the development, progression, recurrence, and propagation of cancers. Accordingly, it will be recognised that this anti-CSC activity of NUC-1031 yields benefits in contexts in which new and effective therapies have long been sought.

DISCLOSURES NOT PART OF THE PRESENT INVENTION

[0012] Also disclosed herein is the use of NUC-1031 in the manufacture of a medicament for targeting cancer stem cells.

[0013] Also disclosed herein is a method of targeting cancer stem cells, the method comprising providing a population of cancer stem cells with an amount of NUC-1031 sufficient to target such cancer stem cells.

[0014] The targeting of cancer stem cells as disclosed herein may be employed in the prevention or treatment of cancer. In methods such as those disclosed herein a population of cancer stem cells may be in a cancer or pre-cancerous condition in a patient in need of such targeting, and the method may comprise administering a therapeutically effective amount of NUC-1031 to the patient.

[0015] Also disclosed herein is NUC-1031 for use as an anti-cancer stem cell medicament. This use of NUC-1031 may also be employed in the prevention or treatment of cancer.

[0016] Also disclosed herein is a method of determining a suitable treatment regimen for a patient with cancer or a pre-cancerous condition, the method comprising:

assaying a biological sample representative of cancer or a pre-cancerous condition in the patient for the presence of CSCs; wherein the presence of CSCs in the biological sample indicates that a suitable treatment regimen will comprise treatment of the patient with NUC-1031.

[0017] Also disclosed herein is NUC-1031 for use in the prevention or treatment of cancer in a

patient selected for such treatment by a method comprising:

assaying a biological sample representative of cancer or a pre-cancerous condition in the patient for the presence of CSCs; wherein the presence of CSCs in the biological sample indicates that the patient is suitable for treatment with NUC-1031.

[0018] The methods disclosed above may further comprise a step of preventing or treating the cancer or pre-cancerous condition using NUC-1031.

[0019] Suitably the cancer is relapsed or refractory cancer. NUC-1031 may be used for the treatment of such relapsed or refractory cancer.

[0020] Also disclosed herein is NUC-1031 for use in treatment of refractory cancer in a human patient.

[0021] Also disclosed herein is the use of NUC-1031 in the manufacture of a medicament for the treatment of relapsed or refractory cancer in a human patient.

[0022] Also disclosed herein is a method of treating relapsed or refractory cancer in a human patient, the method comprising providing a therapeutically effective amount of NUC-1031 to a patient in need of such treatment.

[0023] Also disclosed herein is NUC-1031 for use in the treatment of cancer, wherein NUC-1031 is for use at dose of between approximately 500 mg/m² and 1000 mg/m² per week in at least one initial cycle of treatment, and then for use at a lower weekly dose in at least one further cycle of treatment. The cancer may be a relapsed or refractory cancer.

DESCRIPTION OF THE DRAWINGS

[0024]

Figure 1 shows the structure of gemcitabine.

Figure 2 shows graphs comparing the cytotoxic effects of NUC-1031 and gemcitabine on primary leukaemia cells, and illustrating the significantly lower LD₅₀ value calculated in respect of NUC-1031.

Figure 3 shows dot plots comparing the cytotoxic effects of NUC-1031 and gemcitabine on leukaemic stem cells, and illustrating that treatment with NUC-1031 preferentially depletes CD34⁺/CD123⁺ cells from within treated cell populations.

Figure 4 is a graph illustrating that NUC-1031 demonstrates significant reductions in

CD34⁺/CD123⁺ leukaemic stem cell viability at concentrations of 1 μ M and 2.5 μ M as compared to gemcitabine.

Figure 5 sets out a graph and table comparing LD₅₀ values generated using isolated Rp- and Sp- isomers of NUC-1031, a mixture of NUC-1031 isomers, or gemcitabine in cultures of KG1a leukaemic stem cells.

Figure 6 shows graphs demonstrating the ability of NUC-1031 to target leukaemic CSCs as compared to cytotoxic activity of gemcitabine.

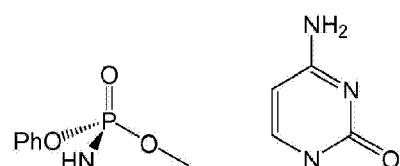
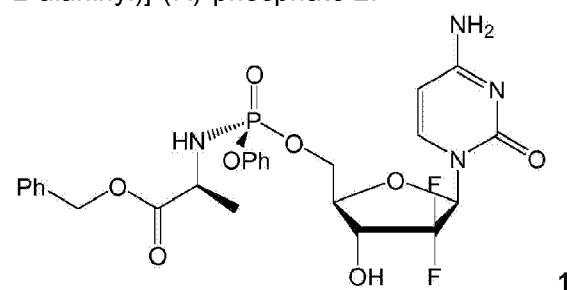
DETAILED DESCRIPTION OF THE INVENTION

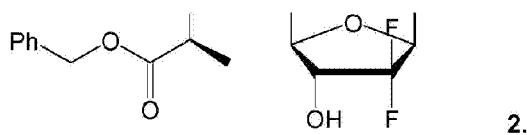
[0025] The following definitions may be useful in the understanding of the invention.

"NUC-1031"

[0026] The compound gemcitabine-[phenyl-benzoxy-L-alaninyl]-phosphate (also referred to as NUC-1031, or by the trade name Acelarin) is a 'ProTide' derivative of the chemotherapeutic agent gemcitabine. It appears to avoid many of the inherent and acquired resistance mechanisms which limit the utility of gemcitabine (see WO2005/012327; and *'Application of ProTide Technology to Gemcitabine: A Successful Approach to Overcome the Key Cancer Resistance Mechanisms Leads to a New Agent (NUC-1031) in Clinical Development'*; Slusarczyk et al.; J. Med. Chem.; 2014, 57, 1531-1542). Conceptually, it is a prodrug of gemcitabine monophosphate, although this does not necessarily reflect its mechanism of action against cancer stem cells.

[0027] NUC-1031 exists in two diastereoisomeric forms, epimeric at the phosphate centre: gemcitabine-[phenyl-benzoxy-L-alaninyl]--(S)-phosphate 1 and gemcitabine-[phenyl-benzoxy-L-alaninyl]--(R)-phosphate 2:





Thus, the NUC-1031 used in the invention may be diastereoisomerically pure or substantially diastereoisomerically pure gemcitabine-[phenyl-benzoxy-L-alaninyl]-(S)-phosphate, it may be diastereoisomerically pure or substantially diastereoisomerically pure gemcitabine-[phenyl-benzoxy-L-alaninyl]-(R)-phosphate or it may be a mixture of the two isomers.

[0028] 'Substantially diastereomerically pure' is defined for the purposes of this invention as a diastereomeric purity of greater than about 90%. It may mean a diastereoisomeric purity of greater than about 95%, greater than about 98%, greater than about 99%, or even greater than about 99.5%. The diastereoisomers may be separated by chromatography (e.g. HPLC, optionally using a chiral column) or they may be separated by crystallisation. It may be more convenient to make a protected form of the NUC1031 diastereoisomeric mixture, to separate the protected forms of the NUC1031 diastereoisomers (e.g. using chromatography or crystallisation) and to subsequently remove the protecting groups to provide the substantially diastereoisomerically pure NUC1031. Alternatively, the diastereoisomers may be synthesised in substantially diastereoisomerically pure form using methods known in the art. This may involve both chemical and enzymatic steps.

[0029] The NUC-1031 may be in the form of a free base or it may be in the form of a pharmaceutically acceptable salt. Suitable pharmaceutically acceptable salts include, but are not limited to, salts of pharmaceutically acceptable inorganic acids such as hydrochloric, sulphuric, phosphoric, nitric, carbonic, boric, sulfamic, and hydrobromic acids, or salts of pharmaceutically acceptable organic acids such as acetic, propionic, butyric, tartaric, maleic, hydroxymaleic, fumaric, malic, citric, lactic, mucic, gluconic, benzoic, succinic, oxalic, phenylacetic, methanesulphonic, toluenesulphonic, benzenesulphonic, salicylic, sulphuric, aspartic, glutamic, edetic, stearic, palmitic, oleic, lauric, pantothenic, tannic, ascorbic and valeric acids. Suitable base salts are formed from bases which form non-toxic salts. Examples include the aluminium, arginine, benzathine, calcium, choline, diethylamine, diolamine, glycine, lysine, magnesium, meglumine, olamine, potassium, sodium, tromethamine and zinc salts. Hemisalts of acids and bases may also be formed, for example, hemisulfate and hemicalcium salts.

[0030] The NUC-1031 may exist in a single crystal form or in a mixture of crystal forms or they may be amorphous. Thus, compounds of the invention intended for pharmaceutical use may be administered as crystalline or amorphous products. They may be obtained, for example, as solid plugs, powders, or films by methods such as precipitation, crystallization, freeze drying, or spray drying, or evaporative drying. Microwave or radio frequency drying may be used for this purpose.

"NUC-1031" formulations

[0031] NUC-1031, or pharmaceutically acceptable salt thereof, may be used alone but will generally be administered in the form of a pharmaceutical composition in which NUC-1031, or pharmaceutically acceptable salt thereof, is in association with a pharmaceutically acceptable adjuvant, diluent or carrier. Conventional procedures for the selection and preparation of suitable pharmaceutical formulations are described in, for example, "Pharmaceuticals - The Science of Dosage Form Designs", M. E. Aulton, Churchill Livingstone, 1988.

[0032] Depending on the mode of administration of NUC-1031, or a pharmaceutically acceptable salt thereof, the pharmaceutical composition which is used to administer NUC-1031, or a pharmaceutically acceptable salt thereof, will preferably comprise from 0.05 to 99 %w (per cent by weight) NUC-1031, or a pharmaceutically acceptable salt thereof, more preferably from 0.05 to 80 %w NUC-1031, or a pharmaceutically acceptable salt thereof, still more preferably from 0.10 to 70 %w NUC-1031, or a pharmaceutically acceptable salt thereof, and even more preferably from 0.10 to 50 %w NUC-1031, or a pharmaceutically acceptable salt thereof, all percentages by weight being based on total composition.

[0033] NUC-1031, or a pharmaceutically acceptable salt thereof, may be administered orally. For oral administration NUC-1031 may be admixed with an adjuvant or a carrier, for example, lactose, saccharose, sorbitol, mannitol; a starch, for example, potato starch, corn starch or amylopectin; a cellulose derivative; a binder, for example, gelatine or polyvinylpyrrolidone; and/or a lubricant, for example, magnesium stearate, calcium stearate, polyethylene glycol, a wax, paraffin, and the like, and then compressed into tablets. If coated tablets are required, the cores, prepared as described above, may be coated with a concentrated sugar solution which may contain, for example, gum arabic, gelatine, talcum and titanium dioxide. Alternatively, the tablet may be coated with a suitable polymer dissolved in a readily volatile organic solvent.

[0034] Preferably, however, NUC-1031 is administered parenterally, and in particular, intravenously. For parenteral (e.g. intravenous) administration NUC-1031, or a pharmaceutically acceptable salt thereof, may be administered as a sterile aqueous or oily solution. NUC-1031 is very lipophilic. Aqueous formulations for intravenous administration, particularly those of the free base of NUC-1031, will typically, therefore, also contain a pharmaceutically acceptable polar organic solvent, e.g. dimethylacetamide, and one or more solubilisers or other additives.

"Cancer stem cells"

[0035] Cancer stem cells, which are sometimes otherwise referred to as "tumour initiating cells", are well known to those skilled in the art. As used herein, the term "cancer stem cell" (normally abbreviated to CSC in the present disclosure) is to be interpreted in accordance with its widely accepted meaning, which is a cell that possesses the capacity to self-renew through asymmetric division, to initiate tumour formation, and to give rise to more mature non-stem cell cancer progeny by differentiation.

[0036] CSCs play a major role in the development, progression, recurrence and propagation of cancers. Accordingly, the finding that NUC-1031 is able to target CSCs, and thereby reduce their numbers, offers therapeutic possibilities in treating these activities.

[0037] As discussed in more detail elsewhere in the specification, CSCs are found in pre-cancerous conditions, where their presence is believed to contribute to the development of such conditions into cancers. As disclosed herein methods of treatment and medical uses, in which NUC-1031 is used to target CSCs, may be used to reduce CSC numbers in pre-cancerous conditions (such as myelodysplastic syndrome, or other conditions considered elsewhere in the specification), and thus to prevent progression of such pre-cancerous conditions into cancer.

[0038] As referred to above, asymmetric cell division of CSCs gives rise to differentiated non-stem cancer cells. The accumulation of such non-stem cancer cells plays a major role in the progression of cancers. Targeting of CSCs by NUC-1031 is able to reduce CSC numbers, which in turn reduces the number of non-stem cancer cell progeny. Thus methods of treatment and medical uses of NUC-1031 in accordance with the present invention are of benefit in treating cancer by preventing cancer progression. Such embodiments are described in more details elsewhere in the present specification.

[0039] CSCs are also able to act as a reservoir of cancer cells that they may cause the recurrence of cancer after remission. Even in the event that the majority of a patient's cancer cells have been removed (for example by surgery, radiotherapy, or chemotherapy, either alone or in combination), so that no observable signs of a cancer remain, the continued presence of CSCs may nucleate the recurrence of the cancer over time. Targeting of CSCs by NUC-1031 provides a new mode by which CSC numbers may be reduced and CSCs killed. Accordingly, and as discussed in more detail elsewhere in the specification, in suitable embodiments the present invention provides methods and medical uses in which NUC-1031 prevents or delays recurrence of cancer.

[0040] Furthermore, movement of CSCs from the site of a cancer to another location within the body can contribute to propagation of cancer, for example by giving rise to metastases. Consequently, the ability of NUC-1031 to target CSCs therefore provides new methods of treatment and medical uses in preventing or treating cancer propagation.

[0041] In addition to their biological activities, CSCs may be identified by their expression of certain characteristic cell surface markers. Cancer stem cells identified in haematological malignancies are typically CD34⁺, while in solid tumours, CD44⁺, CD133⁺ and CD90⁺ have been identified as cancer stem cell markers. The following table summarises examples of known CSC surface phenotypes. It is expected that each of these forms of CSC can be targeted using NUC-1031 in accordance with the invention, and so methods or uses employing NUC-1031 may be used in the treatment of cancers associated with CSCs expressing any of these sets of markers.

Tumour type	CSC Cell surface markers
<i>Solid Tumours</i>	
Breast	CD44 ⁺ /CD24 ^{-/low} /Lineage ⁻ /ESA ⁺
CNS	CD133 ⁺
Colon	CD133 ⁺
Colon	ESA ^{high} /CD44 ⁺ /Lineage ⁻ /(CD166 ⁺)
Ewing's	CD133 ⁺
Head and Neck	CD44 ⁺ /Lineage ⁻
Melanoma	ABCB5 ⁺
Liver	CD90 ⁺ /CD45 ⁻ /(CD44 ⁺)
Cholangiocarcinoma	CD44 ⁺ /GLI1 ⁺ (Glioma-associated oncogene homolog-1)
Ovarian	CD44 ⁺ /CD117 ⁺
Pancreas	CD44 ⁺ /CD24 ⁺ /ESA ⁺
Pancreas	CD133 ⁺
Non-small-cell lung cancer	CD44 ⁺ /Ber-EP4 ⁺
Bladder cancer	CD44 ⁺ /ALDH1A1 ⁺
<i>Haematological tumours</i>	
Acute myeloid leukaemia	Lin ⁻ /CD34 ⁺ /CD38 ⁻ /CD123 ⁺
B-Acute lymphoblastic leukaemia	CD34 ⁺ /CD10 ⁻ or CD34 ⁺ /CD19 ⁻
B-Acute lymphoblastic leukaemia	CD34 ⁺ /CD38 ⁻ /CD19 ⁺
Multiple myeloma	CD34 ⁻ /CD138 ⁻
T-Acute lymphoblastic leukaemia	CD34 ⁺ /CD4 ⁻ or CD34 ⁺ /CD7 ⁻

[0042] The data presented in the Examples demonstrate that NUC-1031 is able to target CSCs of leukaemic stem cell lines, specifically CSCs present in the acute myeloid leukaemia cell line KG1a. This cell line manifests a minor stem cell-like compartment with a distinct immunophenotype (Lin⁻/CD34⁺/CD38⁻/CD123⁺) which is targeted by NUC-1031. Accordingly, methods of treatment or medical uses of NUC-1031 in accordance with the present disclosure may be used to treat leukaemia or other cancers associated with CSCs expressing these characteristic markers.

[0043] The present disclosure also provides methods and medical uses in which patients are selected for treatment of cancer, utilising NUC-1031 in accordance with the present invention, on the basis of the identification of the presence of CSCs in a biological sample representative of the patient's cancer or pre-cancerous condition. The markers set out above provide suitable examples that can be used to identify the presence of CSCs in accordance with such embodiments of the invention. Suitable techniques by which expression of these markers may be investigated in a biological sample are considered further elsewhere in this specification.

"Targeting of cancer stem cells"

[0044] The present invention provides the first indication that NUC-1031 can be used for targeting CSCs in treating cancer. NUC-1031's ability to target CSCs is illustrated in Study 1 set out in the Examples disclosed in this specification.

[0045] It can be seen from the results of Study 1 that when NUC-1031 is provided to populations of cancer cells containing CSCs it targets the CSCs present, leading to a reduction in the total number of cancer cells and in the proportion of total cancer cells exhibiting phenotypic markers of CSCs.

[0046] Without wishing to be bound by any hypothesis, the inventors believe that the reduction in CSC numbers arises as a result of targeted killing of the CSCs among the cancer cell population. That is to say, that NUC-1031 appears to kill CSCs preferentially as compared to killing of non-stem cancer cells, thereby causing the death of CSCs, and a reduction of the proportion of CSCs among the total cancer cell population.

[0047] While the inventors believe that NUC-1031 preferentially kills CSCs as compared to non-stem cancer cells, other mechanisms may also contributed to the reduction in the proportion of CSCs caused by NUC-1031's targeting of these cells.

[0048] Merely by way of example, treatment with NUC-1031 may cause an increase in CSC differentiation, thereby reducing CSC numbers and also the proportion of total cancer cells represented by CSCs. Alternatively, NUC-1031 may cause CSCs to lose their stem cell phenotype, for example losing their ability to self-renew, thereby reducing CSC numbers.

[0049] References to targeting of CSCs in the present disclosure should be interpreted accordingly. For the purposes of the present disclosure, "targeting" of CSCs may be taken as encompassing any mechanism by which NUC-1031 reduces the proportion of CSCs present in a population of cells, whether *in vitro* or *in vivo*. In particular targeting of CSCs may be taken as encompassing preferential killing of CSCs as compared to other cell types, particularly as compared to non-stem cancer cells.

"Prevention or treatment of cancer"

[0050] Disclosed herein are medical uses and methods of treatment in which NUC-1031 is used for the prevention or treatment of cancer. In the context of the present disclosure, "prevention" of cancer is to be considered as relating to prophylactic applications of NUC-1031 used before the development of cancer, and with an aim of stopping cancer from developing. On the other hand "treatment" of cancer is taken as concerning the use of NUC-1031 after cancer has occurred, with a view to ameliorating cancer by slowing or stopping cancer cell proliferation and tumour growth. Advantageously treatment of cancer may cause partial or total reduction in cancer cell numbers and tumour size. Effective treatment of cancer may bring about disease that either "stabilizes" or "responds" in accordance with the RECIST (Response Evaluation Criteria In Solid Tumours) rules.

[0051] As described in more detail below, prevention of cancer may be of particular benefit in patients who have a pre-cancerous condition that increases their likelihood of developing cancer.

"Prevention of cancer"

[0052] Prevention of cancer in accordance with the present disclosure may be effected by treatment of a pre-cancerous condition using NUC-1031 in accordance with the various aspects or embodiments of the invention described herein.

[0053] In particular, prevention of cancer, in the context of the present disclosure, may be achieved by the methods or medical uses disclosed in which NUC-1031 is provided to a patient with a pre-cancerous condition. Methods of treatment or medical uses in accordance with this disclosure may prevent development of the treated pre-cancerous condition into cancer, thereby providing effective prevention of cancer.

[0054] References to prevention of cancer in the context of the present disclosure may also encompass other prophylactic applications of NUC-1031. For example, the ability of NUC-1031 to target CSCs and thereby prevent the development of cancer, and/or prevent the progression of cancer, and/or prevent the recurrence of cancer, and/or prevent the propagation of cancer.

"Pre-cancerous conditions"

[0055] Cancer is frequently preceded by the development of a pre-cancerous condition, which is not itself cancerous, but is associated with an increased risk of cancer. Accumulation of genetic or epigenetic changes may cause previously normal cells to develop a CSC phenotype. Accordingly, CSCs may also be present in such pre-cancerous conditions, as well as in

cancerous conditions.

[0056] It is believed that the presence of CSCs in pre-cancerous conditions contributes to the development of these conditions into cancer. The methods and medical uses of the invention may be employed to target CSCs present in pre-cancerous conditions, and thereby treat such conditions. It will be appreciated that the new and unexpected finding that NUC-1031 targets CSCs means that NUC-1031-treatment of pre-cancerous conditions may be used to prevent such conditions developing into cancer. This represents a way in which NUC-1031 can be used medically in the prevention of cancer, as considered elsewhere in this specification.

[0057] Examples of pre-cancerous conditions include, but are not limited to, those selected from the group consisting of: actinic keratosis, Barrett's oesophagus, dyskeratosis congenital, Sideropenic dysphagia, Lichen planus, oral submucous fibrosis, solar elastosis, cervical dysplasia, leukoplakia, erythroplakia, monoclonal gammopathy of unknown significance (MGUS), monoclonal B-cell lymphocytosis (MBL), myelodysplastic syndromes, as well as pre-cancerous conditions of the stomach such as atrophic gastritis, gastric ulcer, pernicious anaemia, gastric stumps, gastric polyps, and Ménétrier's disease. Among the listed pre-cancerous conditions of the stomach, atrophic gastritis, pernicious anaemia, gastric stumps, and certain types of gastric polyp may have particularly heightened risk of developing into cancers.

[0058] Pre-cancerous conditions often take the form of lesions comprising dysplastic or hyperplastic cells. Accordingly, the presence of dysplasia or hyperplasia, as an alternative or addition to the presence of cells with expressed markers or phenotypes characteristic of CSCs, may be used in the identification of pre-cancerous conditions.

[0059] The severity of dysplasia can vary between different pre-cancerous conditions, or with the development of a single pre-cancerous condition over time. Generally, the more advanced dysplasia associated with a pre-cancerous condition is, the more likely it is that the pre-cancerous condition will develop into cancer. Dysplasia is typically classified as mild, moderate or severe. Severe dysplasia usually develops into cancer if left untreated. Suitably, the methods of treatment of medical uses of the invention employing NUC-1031 may therefore be used to treat a patient with a pre-cancerous condition associated with severe dysplasia. In accordance with the present disclosure NUC-1031 is used to treat a patient with severe cervical dysplasia. Severe cervical dysplasia may be diagnosed by means of a smear test. In further disclosure NUC-1031 is used to treat severe oesophageal dysplasia ("Barrett's oesophagus"). Severe oesophageal dysplasia may be diagnosed following a tissue biopsy.

[0060] It has recently been reported that pre-malignancies can also be identified by detecting somatic mutations in cells in individuals not known to have cancer. In particular, it has been reported that age-related clonal haematopoiesis is a common pre-malignant condition that is associated with increased overall mortality and increased risk of cardiometabolic disease. The majority of mutations detected in blood cells occurred in three genes: DNMT3A, TET2, and ASXL1. Accordingly, patients that will benefit from the use of NUC-1031 to target CSCs, and

thereby treat a pre-cancerous condition, may be identified by assaying a sample comprising blood cells for the presence of genetic mutations indicative of a pre-cancerous condition in at least one of: DNMT3A and/or TET2 and/or ASXL1.

[0061] Pre-cancerous conditions that may benefit from treatment with NUC-1031 in accordance with the disclosure to target CSCs may also be identified by determination of the presence of CSCs with reference to any of the techniques based upon expression of markers characteristic of CSCs, or CSC phenotypes, discussed elsewhere in the specification.

"Treatment of cancer"

[0062] The skilled person will appreciate that there are many measurements by which "treatment" of cancer may be assessed. Merely by way of example, any reduction or prevention of cancer development, cancer progression, cancer recurrence, or cancer propagation may be considered to indicate effective treatment of cancer.

[0063] In certain embodiments, NUC-1031 may be used: to reduce the proportion of CSCs in a population of cancer cells; and/or to inhibit tumour growth; and/or to reduce tumourigenicity; and/or to treat a primary cancer; and/or to treat a relapsed cancer; and/or to treat a metastatic or secondary cancer; and/or to treat, prevent or inhibit metastasis or recurrence; and/or to treat or prevent refractory cancer.

[0064] The ability of cancer treatment using NUC-1031 to bring about a reduction in tumour size, and also to maintain the reduction in tumour size during/after the period in which the treatment is administered represents a particularly relevant indication of effective cancer treatment. As set out in the Examples, the treatments or medical uses of the invention have proven surprisingly effective in this respect, even in the treatment of relapsed or refractory cancers that have previously been resistant to treatment with other therapies.

[0065] The data presented in the Examples illustrate that treatment with NUC-1031 reduces the proportion of CSCs in a population of cancer cells. Characteristic biological activities or cell surface markers by which CSCs may be identified are described elsewhere in the specification. In a suitable embodiment, treatment of cancer in accordance with the present invention may give rise to a reduction in the proportion of CSCs present in a patient's cancer of at least 10%, at least 20%, at least 30%, or at least 40%. In suitable embodiments treatment of cancer in accordance with the invention may give rise to a reduction in the proportion of CSCs present in a patient's cancer of at least 50%, at least 60%, at least 70%, or at least 80%. Treatment of cancer in accordance with the invention may give rise to a reduction in the proportion of CSCs present in a patient's cancer of at least 85%, at least 90%, or at least 95%. Indeed, treatment of cancer in accordance with the invention may give rise to a reduction in the proportion of CSCs present in a patient's cancer of at least 96%, at least 97%, at least 98%, at least 99%, or even 100% (such that substantially no CSCs remain).

[0066] Asymmetric division of CSCs contributes to the growth of tumours. Treatment of cancer with NUC-1031 in accordance with the present invention may bring about an inhibition of tumour growth of at least 10%, at least 20%, at least 30%, or at least 40%. Suitably treatment of cancer in accordance with the invention may give rise to an inhibition of tumour growth of at least 50%, at least 60%, at least 70%, or at least 80%. Treatment of cancer in accordance with the invention may give rise to an inhibition of tumour growth of at least 85%, at least 90%, or at least 95% in a patient so treated. Indeed, treatment of cancer in accordance with the invention may give rise to an inhibition of tumour growth of at least 96%, at least 97%, at least 98%, at least 99%, or even 100% in a treated cancer.

[0067] Tumour growth may be assessed by any suitable method in which the change in size of a tumour is assessed over time. Suitably the size of a tumour prior to cancer treatment may be compared with the size of the same tumour during or after cancer treatment. A number of ways in which the size of a tumour may be assessed are known. For example, the size of a tumour may be assessed by imaging of the tumour *in situ* within a patient. Suitable techniques, such as imaging techniques, may allow the volume of a tumour to be determined, and changes in tumour volume to be assessed.

[0068] As shown in the results set out in the Examples of this specification, the methods of treatment and medical uses of NUC-1031 of the invention are able not only to arrest tumour growth, but are actually able to bring about a reduction in tumour volume in patients with cancers, including patients with relapsed or refractory cancers. Suitably treatment of cancer in accordance with the present invention may give rise to a reduction in tumour volume of at least 10%, at least 20%, at least 30%, or at least 40%. In suitable embodiments, treatment of cancer in accordance with the invention may give rise to a reduction in tumour volume of at least 50%, at least 60%, at least 70%, or at least 80%. Treatment of cancer in accordance with the invention may give rise to a reduction in tumour volume of at least 85%, at least 90%, or at least 95%. Indeed, treatment of cancer in accordance with the invention may give rise to a reduction in tumour volume of at least 96%, at least 97%, at least 98%, at least 99%, or even 100%.

[0069] A reduction in tumour volume of the sort described above can be calculated with reference to a suitable control. For example in studies carried out *in vitro*, or *in vivo* in suitable animal models, the reduction in tumour volume may be determined by direct comparison between the volume of a tumour treated with NUC-1031 and the volume of a control tumour (which may be untreated, or may have received treatment other than with NUC-1031). It will be appreciated that such models requiring lack of treatment of a tumour may not be ethically acceptable in the context of clinical trials or therapeutic management of patients, and in this case a reduction in tumour volume may be assessed by comparing the volume of a treated tumour with the volume of the same tumour prior to treatment, or with a predicted volume that would have been attained by the tumour had no treatment been administered.

[0070] The results set out in the Examples demonstrate that the methods of treatment and medical uses of NUC-1031 of the invention are able to bring about a reduction in biomarkers

indicative of cancer. The reduction of such biomarkers provides a further assessment by which effective treatment of cancer may be demonstrated. Suitable examples of such biomarkers may be selected on the basis of the type of cancer to be treated: in the case of gynaecological cancers CA125 represents a suitable example of a biomarker, while in the case of pancreatic or biliary cancers CA19.9 represents a suitable example of a biomarker, and in the case of colorectal cancers CEA may be a suitable biomarker.

[0071] Suitably treatment of cancer in accordance with the present invention may give rise to a reduction in cancer biomarkers of at least 10%, at least 20%, at least 30%, or at least 40%. In suitable embodiments, treatment of cancer in accordance with the invention may give rise to a reduction in cancer biomarkers of at least 50%, at least 60%, at least 70%, or at least 80%. Treatment of cancer in accordance with the invention may give rise to a reduction in cancer biomarkers of at least 85%, at least 90%, or at least 95%. Indeed, treatment of cancer in accordance with the invention may give rise to a reduction in cancer biomarkers of at least 96%, at least 97%, at least 98%, at least 99%, or even 100%.

[0072] Beneficial effects, such as a reduction in the proportion of CSCs present, reduction in tumour growth, or reduction in tumour volume or cancer biomarkers, observed on treatment of cancer in accordance with the present invention may be maintained for at least one month. Suitably such beneficial effects may be maintained for at least two months, at least three months, at least four months, at least five months, or at least six months. Indeed, such beneficial effects may be maintained for at least 12 months, at least 18 months, or at least 24 months. Suitably the beneficial effects may be maintained for at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, or for ten years or more.

[0073] As disclosed herein, NUC-1031 may be used in a method of preventing or treating cancer or a pre-malignant condition, by targeting cancer stem cells. Also disclosed herein is the use of NUC-1031 in a method of preventing or treating cancer or a pre-malignant condition, wherein the method reduces the tumourigenicity of one or more cancer stem cells. Suitably such methods may prevent the progression of cancer, or inhibit tumour growth.

[0074] When NUC-1031 is used in methods or medical uses of the present disclosure to prevent or treat the progression of a cancer, such prevention or treatment may cause the cancer progression to be slowed, delayed or stopped entirely.

[0075] The progress of a cancer is typically determined by assigning a stage to the cancer. Staging is usually carried out by assigning a number from I to IV to the cancer, with I being an isolated cancer and IV being a cancer that has spread to the limit of what the assessment measures. Specifics of staging vary between cancers, but the stage generally takes into account the size of a tumour, whether it has invaded adjacent organs, how many regional (nearby) lymph nodes it has spread to (if any), and whether it has appeared in more distant locations (metastasised).

[0076] Generally, Stage I is localised to one part of the body and may be treated by surgical resection (for solid tumours that are small enough). Stage II is locally advanced, and is treatable by chemotherapy, radiation therapy, surgery, or a combination thereof. Stage III is also locally advanced and the designation of Stage II or Stage III depends on the specific type of cancer, although Stage III is generally accepted to be "late" locally advanced. Stage IV cancers have often metastasised to a second organ. Treatment of cancer using NUC-1031 in the methods or medical uses of the present invention may be used to treat a stage I, II, III or IV cancer by targeting CSCs. Treatment with NUC-1031 may be used to prevent the progression of a cancer from one stage to the next. In one embodiment, treatment with NUC-1031 is used to prevent progression from Stage I to Stage II. In another embodiment, treatment with NUC-1031 is used to prevent progression from Stage II to Stage III. In still another embodiment, treatment with NUC-1031 is used to prevent progression from Stage III to Stage IV.

[0077] Preventing or inhibiting progression of the cancer is particularly important for preventing the spread of the cancer, for example the progression from Stage I to Stage II where the cancer spreads locally, or the progression from Stage III to Stage IV where the cancer metastasises to other organs. CSCs are tumourigenic and so are believed to play a critical role in the spread of cancer, both locally and metastatically. Methods of treatment or medical uses of the invention employing NUC-1031 can therefore be used to prevent the spread of cancer, by targeting tumourigenic CSCs and thus reducing their numbers.

"Cancers"

[0078] CSCs play a role in the biological activity of a wide range of cancers. Accordingly, there are a wide range of cancers that may be treated in accordance with the present invention.

[0079] As discussed elsewhere herein, CSCs are known to be present in many tumour types including liquid tumours (including haematological tumours such as leukaemias and lymphomas) and solid tumours (such as breast, lung, colon, prostate, ovarian, skin, bladder, biliary and pancreas tumours). Methods of treatment and medical uses of NUC-1031 to target CSCs are therefore expected to be useful in the treatment of such cancers.

[0080] Suitably NUC-1031 may be used in the treatment of a cancer selected from the group consisting of: leukaemia, lymphoma, multiple myeloma, lung cancer, liver cancer, breast cancer, head and neck cancer, neuroblastoma, thyroid carcinoma, skin cancer (including melanoma), oral squamous cell carcinoma, urinary bladder cancer, Leydig cell tumour, biliary cancer, such as cholangiocarcinoma or bile duct cancer, pancreatic cancer, colon cancer, colorectal cancer and gynaecological cancers, including ovarian cancer, endometrial cancer, fallopian tube cancer, uterine cancer and cervical cancer, including epithelia cervix carcinoma. In suitable embodiments, the cancer is leukaemia and can be selected from the group consisting of acute lymphoblastic leukaemia, acute myelogenous leukaemia (also known as acute myeloid leukaemia or acute non-lymphocytic leukaemia), acute promyelocytic leukaemia, acute lymphocytic leukaemia, chronic myelogenous leukaemia (also known as chronic myeloid

leukaemia, chronic myelocytic leukaemia or chronic granulocytic leukaemia), chronic lymphocytic leukaemia, monoblastic leukaemia and hairy cell leukaemia. In further preferred embodiments, the cancer is acute lymphoblastic leukaemia. In a suitable embodiment the cancer is lymphoma, which may be selected from the group consisting of: Hodgkin's lymphoma; non-Hodgkin lymphoma; Burkitt's lymphoma; and small lymphocytic lymphoma.

[0081] Suitably targeting CSCs in such cancers may achieve effective treatment of the cancer by preventing or treating the development of the cancer, by preventing or treating the progression of the cancer, by preventing or treating the recurrence of the cancer, or by preventing or treating the propagation of the cancer.

[0082] In a suitable embodiment the present invention provides NUC-1031 for use in targeting CSCs in the treatment of metastatic cancer.

[0083] In a suitable embodiment the present invention provides NUC-1031 for use in targeting CSCs in the treatment of relapsed or refractory cancer.

[0084] In a suitable embodiment the present invention provides NUC-1031 for use in targeting CSCs in the treatment of a primary cancer. Suitably the primary cancer treated may be a second primary cancer.

The invention provides NUC-1031 for use in targeting CSCs in the treatment of secondary cancer. In a suitable embodiment the secondary cancer is a metastatic cancer.

[0085] In a suitable embodiment the present invention provides NUC-1031 for use in targeting CSCs, wherein the targeting of CSCs prevents or inhibits: (i) recurrence of a cancer; (ii) occurrence of second primary cancer; or (iii) metastasis of a cancer.

[0086] Methods of treatment or medical uses in which NUC-1031 is employed on the basis of its ability to target CSCs may be used in the treatment of relapsed or refractory cancer. The considerations regarding relapsed or refractory cancer in such embodiments are, except for where the context requires otherwise, the same as for the treatment of relapsed or refractory cancer in connection with the eighth to tenth aspects of the invention.

"Relapsed or refractory cancer"

[0087] As noted above, certain aspects and embodiments of the invention particularly relate to the use of NUC-1031 in the treatment of relapsed or refractory cancers.

[0088] For the purposes of the present invention, refractory cancers may be taken as cancers that demonstrate resistance to treatment by anti-cancer therapies other than those utilising NUC-1031. For example, NUC-1031 may be used in the treatment of refractory cancers that are resistant to treatment with radiotherapy. Alternatively, or additionally, NUC-1031 may be used in the treatment of refractory cancers that are resistant to biological agents used in the

treatment of cancer. In a suitable embodiment NUC-1031 may be used in the treatment of refractory cancers that are resistant to treatment with chemotherapeutic agents other than NUC-1031.

[0089] In particular, refractory cancers that may benefit from the methods of treatment or medical uses of the invention employing NUC-1031 include those cancers that are resistant to gemcitabine.

[0090] Relapsed cancers (or recurrent cancers) are those that return after a period of remission during which the cancer cannot be detected. Cancer recurrence may occur at the site of the original cancer (local cancer recurrence), at a site close to that of the original cancer (regional cancer recurrence), or at a site distant from that of the original cancer (distal cancer recurrence). CSCs are believed to play a role in the recurrence of cancer, providing a source from which cells of the relapsed cancer are generated. Accordingly, the methods of treatment and medical uses of NUC-1031 in accordance with the invention, which enable targeting of CSCs, may be of great benefit in the context of relapsed cancers. The ability of NUC-1031 to target CSCs may be used to remove the populations of such cells that are able to give rise to recurrence, thus preventing incidences of relapsed cancer. The anti-CSC activity of NUC-1031 may also be used to target CSCs in cancers that have recurred, as well as potentially exerting cytotoxic effects on non-stem cancer cells, thereby providing treatment of relapsed cancers.

[0091] In view of the above, it will be appreciated that NUC-1031 may be used in the methods or uses of the invention for the treatment of a relapsed cancer. NUC-1031 may be used in the methods or uses of the invention for the treatment of a local, regional or distant relapsed cancer.

[0092] NUC-1031 may be used in the methods or uses of the invention to prevent the recurrence of cancer by providing at least 2 months, at least 6 months, at least 12 months, at least 18 months, at least 24 months, or at least 30 months of remission. Indeed, NUC-1031 may be used to prevent recurrence of cancer by providing at least 4 years, at least 5 years, at least 6 years, at least 7 years, at least 8 years, at least 9 years, or at least 10 years of remission.

[0093] NUC-1031 may be used in the methods or uses of the invention to treat a relapsed cancer which has recurred after at least 2 months, at least 6 months, at least 12 months, at least 18 months, at least 24 months, or at least 30 months of remission. Indeed, NUC-1031 may be used to treat a relapsed cancer which has recurred after at least 4 years, at least 5 years, at least 6 years, at least 7 years, at least 8 years, at least 9 years, or at least 10 years of remission.

[0094] The ability of NUC-1031 to target CSCs gives rise to the ability of this compound to treat cancers in accordance with the medical uses or methods of treatment of the first to sixth aspects of the invention. However, it should be noted that NUC-1031 also exerts a direct cytotoxic effect upon non-stem cancer cells that make up the bulk of tumours. While activity of

CSCs may underlie much of the resistance that makes relapsed or refractory cancers so difficult to treat, non-stem cancer cells are also a major constituent of such relapsed or refractory cancers.

[0095] NUC-1031 exerts greater cytotoxic effects on non-stem cancer cells than does gemcitabine, the chemotherapeutic molecule from which NUC-1031 is derived. Accordingly, the mechanism by which NUC-1031 acts in the treatment of relapsed or refractory cancer, for example in the eighth, ninth, or tenth aspects of the invention may not be limited solely to the anti-CSC activity of this compound, but may also make use of the action of NUC-1031 on non-stem cancer cells. In such uses treatment with NUC-1031 will reduce the total number of both CSCs and non-stem cancer cells, but will preferentially reduce the proportion of CSCs that remain after treatment.

Therapeutically effective doses of NUC-1031

[0096] A therapeutically effective amount of NUC-1031 may be an amount sufficient to induce death of CSCs. In some embodiments, particularly those relating to the treatment of relapsed or refractory cancer, a therapeutically effective amount of NUC-1031 may be an amount sufficient to induce death of CSCs and also to induce death of non-stem cancer cells.

[0097] There are various different ways in which the amount of a therapeutically effective compound, such as NUC-1031, to be administered to a patient may be calculated and expressed. One such way which is considered particularly relevant in doses of agents for the treatment of cancer, is in the amount of the agent to be administered per unit of body surface area of the patient. Such doses are typically expressed in terms of the amount of the agent (which may be determined by mass) per square meter (m^2) of surface area.

[0098] Uses of NUC-1031 for the treatment of cancer may utilise a weekly dose of between 250 mg/ m^2 and 1000 mg/ m^2 . Such treatments may, for example utilise a weekly dose of between 375 mg/ m^2 and 900 mg/ m^2 . As described further in the Examples, the inventors have found that NUC-1031 achieves effective treatment of relapsed or refractory cancers when patients are provided with weekly doses ranging between approximately 500 mg/ m^2 and 825 mg/ m^2 .

[0099] Without wishing to be bound by any hypothesis, the inventors believe that the ability of NUC-1031 to target CSCs allows therapeutic effectiveness to be achieved using lower doses of this compound than would otherwise be expected. Merely by way of example, weekly doses of NUC-1031 that are as low as 825 mg/ m^2 , 750 mg/ m^2 , 600 mg/ m^2 , or 500 mg/ m^2 may prove therapeutically effective in the uses and methods of the invention.

[0100] A chosen weekly dose of NUC-1031 may be provided in a single incidence of

administration, or in multiple incidences of administration during a week. For example, a weekly dose of NUC-1031 may be provided in two incidences of administration, in three incidences of administration, or more. Thus, in the case of a weekly dose of 750 mg/m², this may be achieved by three administrations of 250 mg/m² over the course of a week, or two administrations of 375 mg/m² during a week. Similarly, in the case of a weekly dose of 600 mg/m², this may be achieved by three administrations of 200 mg/m² over the course of a week, or two administrations of 300 mg/m² during a week.

[0101] A suitable amount of NUC-1031 to be administered in a single incidence of treatment in order to provide a required dose of this compound over the course of week may be between approximately 100 mg/m² and 300 mg/m².

[0102] The weekly dose of NUC-1031 provided may decrease over the course of treatment. For example, the inventors have found that treatment may be started at a weekly dose of around 1000 mg/m², 900 mg/m², 825 mg/m², 750 mg/m², or 725 mg/m². Over the course of treatment the dose needed may decrease to around 750 mg/m² (in cases where the initial dose is above this amount), around 650 mg/m², around 625 mg/m², or even around 500 mg/m² or around 375 mg/m².

[0103] Doses of therapeutic agents such as NUC-1031 can, of course, be presented in other manners. The most common of these is the amount of the active agent to be provided per unit body mass. It has been calculated that for an average human patient a dose of 1 mg/m² is equivalent to approximately 0.025 mg/kg body mass. Accordingly, the data indicate that NUC-1031 is effective for the treatment of relapsed or refractory cancer at doses ranging from approximately 6.25 mg/kg to approximately 25 mg/kg. A suitable dose may, for example, be of between about 9.5 mg/kg and 22.5 mg/kg. In a suitable embodiment NUC-1031 achieves effective treatment of relapsed or refractory cancers when patients are provided with weekly doses ranging between approximately 12.5 mg/kg and 20.5 mg/kg.

[0104] Considerations regarding formulations of NUC-1031 suitable for use in the methods of treatment and medical uses of the present invention are described elsewhere in this disclosure. In the case of injectable formulations of NUC-1031, these may be administered intravenously. Intravenous administration may be achieved over any suitable time frame, for example in a ten minute injection, or the like.

Dosage regimens

[0105] The eleventh aspect of the invention provides NUC-1031 for use in the treatment of cancer, wherein NUC-1031 is for use at a relatively high dose (of between approximately 625 mg/m² and 1000 mg/m² per week) for at least one cycle at the start of treatment, before

changing to a lower weekly dose in at least one further cycle of treatment. Such dosage regimens may be used in the treatment of cancer by targeting CSCs, or in the treatment of relapsed or refractory cancer.

[0106] In a suitable example, NUC-1031 may be administered as a bolus intravenous injection over a period of 5 minutes, 10 minute, or 30 minutes.

[0107] Suitably NUC-1031 may be administered on days 1, 8, 15 of a 4 weekly Cycle for up to 6 cycles. Alternatively, NUC-1031 may be administered on days 1, 5, 8, 12, 15, 19, of a 4 weekly Cycle for up to 6 Cycles.

[0108] Dosage regimens and cycles providing for twice weekly provision of NUC-1031 are particularly potent in the treatment of cancer, such as relapsed or refractory cancer.

Types of treatment

[0109] In a suitable embodiment the present invention NUC-1031 for use according to claim 1 in targeting CSCs as a first line treatment of cancer.

[0110] However, the finding that NUC-1031 is able to target CSCs and thereby treat relapsed or refractory cancer illustrates that NUC-1031 is able to provide effective treatment of cancer in contexts in which other treatments have proved ineffective. Accordingly, in a suitable embodiment the present invention provides NUC-1031 for targeting CSCs as a second line treatment of cancer. Indeed, in a suitable embodiment the present invention provides NUC-1031 for targeting CSCs as a third, or further, line treatment of cancer.

[0111] In a suitable embodiment the present invention provides NUC-1031 for use as a neoadjuvant in the treatment of cancer. A neoadjuvant is an agent provided to a patient in order to reduce the size of a tumour prior to a "main" anti-cancer therapy, such as surgical removal of cancer. NUC-1031 may be used as a neoadjuvant therapy for a patient who will subsequently undergo surgical treatment of cancer and/or radiotherapy for cancer.

[0112] Alternatively, or additionally, the invention provides NUC-1031 for use as an adjuvant in the treatment of cancer. An adjuvant is an agent provided to a patient after a "main" anti-cancer therapy, such as surgical removal of cancer, in order to prevent the return of cancer after the main therapy. NUC-1031 may be used as an adjuvant for a patient who has undergone surgical treatment of cancer and/or radiotherapy for cancer.

[0113] NUC-1031 may be employed in the methods or uses of the invention in a monotherapy, which is to say in treatments in which NUC-1031 provides substantially all of the therapeutic activity that is made use of in the treatment.

[0114] Alternatively, the methods or uses of the invention may employ NUC-1031 in a

combination therapy. In such embodiments NUC-1031 is used in conjunction with at least one further cancer therapy. The further cancer therapy may comprise surgery and/or radiotherapy. Additionally, or alternatively, the further cancer therapy may comprise use of at least one further therapeutic agent that contributes to the treatment of cancer to be achieved. Suitably such an agent may be a chemotherapeutic agent or a biological agent used in the treatment of cancer.

[0115] In a suitable embodiment of a combination therapy the NUC-1031 and a further therapeutic agent may be provided to a patient at the same time. In a suitable example, the NUC-1031 and a further therapeutic agent may be formulated as part of the same pharmaceutical composition. Alternatively the NUC-1031 and a further therapeutic agent may be formulated separately for provision to the patient at substantially the same time.

[0116] In another suitable embodiment of a combination therapy, the NUC-1031 and a further therapeutic agent may be provided to a patient at different times. The NUC-1031 and a further therapeutic agent may be provided to a patient sequentially. For example, the NUC-1031 may be provided to the patient prior to provision of the further therapeutic agent. Alternatively NUC-1031 may be provided to the patient after provision of the further therapeutic agent.

"Further therapeutic agents"

[0117] NUC-1031 may be used in combination with a wide range of further therapeutic agents for the prevention or treatment of cancer. These include biological agents, immunotherapeutic agents, and chemotherapeutic agents that may be used for the prevention or treatment of cancer.

[0118] While specific examples of suitable further agents are considered in the following paragraphs, these should not be taken as limiting the range of further therapeutic agents suitable for use with NUC-1031. Indeed, the ability of NUC-1031 to target CSCs indicates that it may be beneficially used in combination with any further therapeutic agent used in the prevention or treatment of cancer, whether such further agent targets CSCs, non-stem cancer cells, or other cells or constituents involved in the development, maintenance or propagation of cancer.

[0119] Examples of further therapeutic agents that may be used in combination with NUC-1031 include:

1. (a) an anti-angiogenic agent, optionally wherein the anti-angiogenic agent is: (i) an inhibitor of the VEGF pathway, optionally bevacizumab; (ii) a tyrosine kinase inhibitor, optionally sorafenib, sunitinib or pazopanib; or (iii) an mTOR inhibitor, optionally everolimus;
2. (b) an alkylating agent;
3. (c) an anti-metabolite;

4. (d) an anti-tumour antibiotic;
5. (e) a topoisomerase;
6. (f) a mitotic inhibitor;
7. (g) a monoclonal antibody;
8. (h) a metallic agent; or
9. (i) an active or passive immunotherapy.

[0120] Except for where the context requires otherwise, the further therapeutic agents set out in the preceding list should all be considered suitable for use in any of the embodiments of combination therapies with NUC-1031 considered above.

Selection of patients

[0121] The inventors' finding that NUC-1031 is able to target CSCs makes possible a number of methods by which it is possible to determine whether a particular patient is likely to benefit from receiving NUC-1031 in the treatment of cancer, such as relapsed or refractory cancer.

[0122] Accordingly, the invention provides, a method of determining whether a patient with cancer or a pre-cancerous condition will benefit from treatment of cancer with NUC-1031, the method comprising: assaying a biological sample representative of cancer or a pre-cancerous condition in the patient for the presence of CSCs; wherein the presence of CSCs in the biological sample indicates that the patient will benefit from treatment with NUC-1031.

[0123] Also disclosed herein is a method of determining a suitable treatment regimen for a patient with cancer or a pre-cancerous condition, the method comprising: assaying a biological sample representative of cancer or a pre-cancerous condition in the patient for the presence of CSCs; wherein the presence of CSCs in the biological sample indicates that a suitable treatment regimen will comprise treatment of the patient with NUC-1031.

[0124] Also disclosed herein is NUC-1031 for use in the prevention or treatment of cancer in a patient selected for such treatment by a method comprising: assaying a biological sample representative of cancer or a pre-cancerous condition in the patient for the presence of CSCs; wherein the presence of CSCs in the biological sample indicates that the patient is suitable for treatment with NUC-1031.

[0125] In suitable embodiments CSCs in a biological sample may be identified by their expression of characteristic patterns of markers discussed previously in the application.

[0126] The skilled person will appreciate that there are many suitable examples of biological samples that may be used in embodiments of the invention such as those set out above. Suitably such a sample may include cells from the cancer or pre-cancerous condition. A

suitable biological sample may be a tissue sample, such as a sample for use in histology. Cells in such samples may be directly assessed for their expression of CSC markers, such as those set out above.

[0127] Alternatively or additionally, a suitable biological sample may comprise target molecules representative of gene expression by cells of the cancer or pre-cancerous condition. Examples of such target molecules include proteins encoded by the genes expressed, or nucleic acids, such as mRNA, representative of gene expression.

[0128] Suitable examples of techniques by which expression of CSC markers may be assessed may be selected with reference to the sample type. Techniques for the investigation of expressed markers are frequently used in the context of clinical assessments (such as for diagnostic or prognostic purposes) and their use will be familiar to those required to practice them in the context of the present invention. Merely by way of example, in samples containing proteins the presence of CSC markers may be assessed by suitable techniques using antibodies that react with the CSC markers in question. Examples of such samples containing protein CSC markers include histology samples (where the presence of the markers may be visualised by suitable immunocytochemistry techniques), or samples derived from the circulation. Here the presence of circulating CSCs (which are believed to contribute to the propagation of cancer through metastasis) may be assessed using techniques such as flow cytometry.

[0129] In samples containing nucleic acids representative of expression of CSC markers, such expression may be assessed by suitable molecule biology techniques, such as by polymerase chain reaction (PCR) amplification using suitable primers.

[0130] The invention will now be further described with reference to the following Examples.

EXAMPLES

1 NUC-1031 preferentially targets CSCs

[0131] The following study illustrates the ability of NUC-1031 to preferentially target CSCs *in vitro*, and thereby reduce the proportion of CSCs present in populations of cancer cells.

1.1 Comparison of LD₅₀ values for NUC-1031 and gemcitabine in primary acute myeloid leukaemia blasts

[0132] NUC-1031 was assayed for its cytotoxic effects on primary cultures of acute myeloid leukaemia (AML) blasts. LD₅₀ values (the concentration required to kill 50% of the tumour cells

in culture) were calculated in respect of NUC-1031 and gemcitabine.

In vitro cytotoxicity assay in primary acute myeloid leukemia cells

[0133] Bone marrow samples were collected in ethylenediaminetetraacetic acid (EDTA) from newly diagnosed, previously untreated, acute myeloid leukemia (AML) patients. AML blasts were enriched by density gradient centrifugation using Histopaque (Sigma, Poole, UK) and were subsequently maintained in Roswell Park Memorial Institute medium (RPMI) supplemented with 10% foetal bovine serum (FBS). Cells were treated with Gemcitabine or NUC-1031 at concentrations between 0.25 μ M and 10 μ M, and incubated for 48h. All cultures were maintained at 37°C in a 5% CO₂ humidified atmosphere.

Measurement of *in vitro* apoptosis in primary AML cells

[0134] Cells were harvested and labelled with CD34-fluorescein isothiocyanate (FITC) (BD Biosciences, Buckingham, UK) and then resuspended in 200 μ l of binding buffer containing 4 μ L of annexin V labelled with allophycocyanin (APC) (eBioscience Ltd, Hatfield, UK). Apoptosis was quantified in the CD34⁺ AML cells using an Accuri C6 flow cytometer (Becton Dickinson, CA, USA). At least 10,000 events were acquired and data were subsequently analysed using FlowJo software (Tree Star Inc., Ashland, OR, USA). All LD₅₀ values (concentration of drug required to kill 50% of cells) were derived from the dose-response curves.

Identification and quantification of CD34⁺/CD123⁺ sub-populations in primary AML cells

[0135] Putative leukaemic stem cells were identified by dual expression of CD34 and CD123. The relative sensitivity of these cells to the effects of gemcitabine and NUC-1031 were assessed as a function of the percentage of these cells that remained viable following exposure to molar equivalents of each agent.

[0136] The results of this study are shown in Figure 2, in which panel A shows the overlaid dose-response curves calculated in respect of both NUC-1031 and gemcitabine, and panel B is a bar graph illustrating the significantly lower mean LD₅₀ value obtained in respect of NUC-1031 (1.6 x 10⁻⁶ M) as compared to gemcitabine (3.1 x 10⁻⁶ M).

[0137] In addition to the increased potency exhibited by NUC-1031 when compared with gemcitabine, it also showed an enhanced ability to deplete CSCs in the AML blast cultures at micromolar concentrations (Figures 3 and 4). The cytotoxic activity of NUC-1031 measured in this assay demonstrated increased potency in respect of both CSCs and non-stem cancer cells

when compared with gemcitabine.

1.2 Preferential targeting of CSCs by NUC-1031

[0138] The respective abilities of NUC-1031 and gemcitabine to target CSCs were investigated in the AML cell line KG1a. The KG1a cell line was chosen in particular for this study because CSCs within the population exhibit a Lin⁻/CD34⁺/CD38⁻/CD123⁺ immunophenotype that allows them to readily be distinguished from non-stem cancer cells (also termed "bulk" cancer cells) within the population.

1.3 KG1a cell culture conditions

[0139] The acute myeloid leukaemia (AML) KG1a cell line was maintained in RPMI medium (Invitrogen, Paisley, UK) supplemented with 100 units/ml penicillin, 100µg/ml streptomycin and 20% foetal calf serum. Cells were subsequently aliquoted (10⁵ cells/100µl) into 96-well plates and were incubated at 37°C in a humidified 5% carbon dioxide atmosphere for 72h in the presence of NUC-1031 or gemcitabine at concentrations that were experimentally determined for each compound. In addition, control cultures were carried out to which no drug was added. Cells were subsequently harvested by centrifugation and were analysed by flow cytometry using the Annexin V assay.

1.4 Measurement of in vitro apoptosis

[0140] Cultured cells were harvested by centrifugation and then resuspended in 195µl of calcium-rich buffer. Subsequently, 5 µl of Annexin V (Caltag Medsystems, Botolph Claydon, UK) was added to the cell suspension and cells were incubated in the dark for 10 mins prior to washing. Cells were finally resuspended in 190µl of calcium-rich buffer together with 10µl of propidium iodide. Apoptosis was assessed by dual-colour immunofluorescent flow cytometry as described previously. Subsequently LD₅₀ values (the dose required to kill 50% of the cells in a culture) were calculated for each nucleoside analogue and ProTide.

1.5 Immunophenotypic identification of the leukaemic stem cell compartment

[0141] KG1a cells were cultured for 72 hours in the presence of a wide range of concentrations of each nucleoside analogue and their respective ProTides. Cells were then harvested and labelled with a cocktail of anti-lineage antibodies (PE-cy7), anti-CD34 (FITC), anti-CD38 (PE) and anti-CD123 (PERCP cy5). The sub-population expressing a leukaemic stem cell (LSC) phenotype were subsequently identified and were expressed as a percentage

of all viable cells left in the culture. The percentages of stem cells remaining were then plotted on a dose-response graph and the effects of the ProTides were compared with the parental nucleoside.

[0142] KG1a cells were cultured for 72 hours in the presence of a wide range of concentrations of each compound assayed. Cells were then harvested and labelled with a cocktail of anti-lineage antibodies (PE-cy7), anti-CD34 (FITC), anti-CD38 (PE) and anti-CD123 (PERCP cy5). The sub-population expressing a CSC phenotype were subsequently identified and were expressed as a percentage of all viable cells left in the culture. The percentages of CSCs remaining were then plotted on a dose-response graph and the effects of the NUC-1031 (and its purified isomers) were compared with gemcitabine.

1.6 Statistical analysis

[0143] The data obtained in these experiments were evaluated using one way ANOVA. All data was confirmed as Gaussian or a Gaussian approximation using the omnibus K2 test. LD₅₀ values were calculated from the non-linear regression and line of best-fit analysis of the sigmoidal dose-response curves. All statistical analyses were performed using Graphpad Prism 6.0 software (Graphpad Software Inc., San Diego, CA).

[0144] In KG1a cells, NUC-1031 (and its purified isomers) showed increased *in vitro* potency when compared to Gemcitabine (Figure 5). However, there was no significant difference in potency between the unseparated mixture and the purified isomers of NUC-1031. Furthermore, NUC-1031 showed preferential targeting of CSCs when compared with Gemcitabine. This was consistently observed at sub-micromolar concentrations of ProTide (Figure 6). Again, the two purified isomers of NUC-1031 showed no significant difference in their ability to target CSCs in our experimental system.

2 NUC-1031 is able to treat relapsed or refractory cancers in human patients

[0145] The following data were generated in clinical studies of NUC-1031 in human patients with advance progressive cancers that are refractory to, or have relapsed on, all conventional therapies that have been used to date. The results clearly illustrate the ability of NUC-1031 to successfully treat refractory cancers.

[0146] Although the primary objectives of the dose escalation part of the study were to determine the Recommended Phase II Dose (RP2D) and safety profile, secondary objectives included determining the PK profile, however effective treatment of patients with a range of refractory cancers has also been observed as part of this study.

[0147] A total of 68 patients have been entered into this dose escalation study, of which 49

were evaluable for clinical response in that they have received at least 2 Cycles of NUC-1031 and were therefore eligible for a RECIST 1.1 assessment.

Table 1: Best Overall Response in the ProGem1 Study

Patients	n = 68	Best Overall Response (to date)	
Evaluable	n = 49	Partial Responses	5 (10%)
		Stable Disease	33 (67%)
		Progressive Disease	11 (22%)
Non Evaluable	n = 19		

[0148] NUC-1031 was administered as a 5 to 30 minute intravenous slow bolus injection.

- Schedule A: NUC-1031 was administered on days 1, 8, 15 of a 4 weekly Cycle.
- Schedule B: NUC-1031 was administered on days 1, 5, 8, 12, 15, 19, of a 4 weekly Cycle.

EVALUABLE PATIENTS [n=49]

[0149] Evaluable patients were patients that received \geq 2 Cycles of NUC-1031 and were therefore eligible for a RECIST 1.1 assessment at the end of Cycle 2. Where the disease response duration, measured in months, is followed by a "+" this indicates ongoing disease control as of the latest cut-off date.

Patient 004 Breast Cancer: Stable Disease

Female (67 years)

[0150] Diagnosed with Grade 2 invasive ductal carcinoma of the breast (ER+ve, HER2-ve) in 2002. As first line treatment she was given surgery and received adjuvant epirubicin + docetaxel, radiotherapy and maintenance hormone therapy with tamoxifen, then anastrozole until 2010. In 2010, patient was diagnosed with metastatic disease in the bone and was treated with palliative radiotherapy and commenced on ibandronate and fulvestrant as second line treatment.

[0151] Disease progression was noted in 2012 and she was given third line chemotherapy with capecitabine and navelbine for 4 months, but while on treatment her disease progressed, with new liver and lung metastases. The patient was commenced on a PI3K inhibitor (Phase 1

study) in September 2012 and received 2 Cycles (2 months), but disease progressed, with increase in the size of liver metastases, while on treatment.

[0152] Commenced NUC-1031 on 3rd December 2012 on 500 mg/m² weekly. Completed 6 Cycles and tolerated treatment well. Patient had Stable Disease at end of study and requested compassionate continuation of a 7th Cycle of NUC-1031, then elected for a 'drug holiday' after Cycle 7. Remained stable for further 5 months with no further treatment before disease progression.

[0153] Stable Disease to RECIST (12 months).

Patient 005 Ovarian Cancer: Stable Disease

Female (58 years)

[0154] Diagnosed with Stage 3c (Grade 3) bilateral serous ovarian cancer in 2009. On the 23rd June 2009 the patient had a total abdominal hysterectomy and salpingo-oophorectomy performed, but unresectable omental deposits were left *in situ*.

[0155] As first line chemotherapy, in October 2009 the patient received 6 Cycles of carboplatin + paclitaxel, but developed an allergic reaction to carboplatin at final treatment Cycle.

[0156] First relapse was 8 months later and in December 2010 patient commenced on 6 Cycles of Caelyx plus VEGFR-2 inhibitor (Phase II clinical study), remaining on VEGFR-2 monotherapy as maintenance. Second relapse was 9 months later, with a rise in CA125 and CT evidence of a left sided pelvic mass measuring 3.2cm. Commenced on third line chemotherapy and received 6 Cycles of weekly paclitaxel and there was an initial moderate response with a fall in CA125 and some reduction in tumour volume. Disease progression was confirmed 5 months later with rising CA125 levels and increased tumour size. Patient received the last dose of paclitaxel in March 2012.

[0157] Commenced NUC-1031 on 7th January 2013 on 500 mg/m² weekly. Completed 6 Cycles as per Study protocol, with Stable Disease, and then a further 6 months of NUC-1031, resulting in 12 months of treatment. Patient tolerated treatment well. CA125 levels dropped from 208 at start of study to 140 at the end of Cycle 6. Patient elected to stop therapy after 12 months, and relapsed 3 months after stopping treatment.
Stable Disease to RECIST (15 months).

Patient 006 Cholangiocarcinoma: Stable Disease

Male (43 years)

[0158] Diagnosed with a primary cholangiocarcinoma in 2009. A Whipple procedure was performed and the patient was given 6 cycles of adjuvant gemcitabine. On disease recurrence in February 2012, the patient was commenced on CapeOx until July 2012. Later that year a CT scan showed bone metastases and these were treated with a course of radiotherapy to the lower back.

[0159] Commenced NUC-1031 on 31st January 2013 on 375 mg/m² twice weekly, and completed 2 Cycles. Changed to schedule A at 500 mg/m² and received 1 further dose. Reduction in CA19.9 from 125,002 to 59,285 after only two doses of NUC-1031. CT scan revealed a reduction in metastatic lung lesion and lymph node from baseline.

Stable Disease to RECIST (3 months).

Patient 007 Colorectal Cancer: Stable Disease**Male (73 years)**

[0160] Diagnosed with colorectal cancer in 2008. Following 6 Cycles of FOLFOX had the primary tumour removed in April 2009. Further surgery in August 2009 when a right hepatectomy and ileostomy were performed. In July 2010 was included in the PICCOLO trial (panitumumab + irinotecan). Six months later received radiofrequency ablation (RFA) plus biliary stenting for additional complications. In December 2011 he received 7 Cycles of cetuximab + irinotecan + 5-FU, but with subsequent disease progression.

[0161] Commenced NUC-1031 on 11th February 2013 on 375 mg/m² twice weekly, and completed 0.5 of a Cycle.

Following treatment delays, and at the patient's request for convenience, he was changed to a weekly schedule at 500 mg/m² and completed 2 Cycles. Over the treatment period he developed thrombocytopaenia (G3) (possible linked to splenomegaly seen at baseline). An ultrasound scan on 25th February 2013 showed compression of portal vein, with possible thrombosis, as a result of the splenomegaly. Reduction in CEA from 361 to 286 and CA19.9 from 3,151 to 2,957.

Stable Disease to RECIST (3 months).

Patient 008 Cancer of Unknown Primary: Stable Disease

Female (37 years)

[0162] Diagnosed with a retroperitoneal mass and metastatic disease of unknown primary in 2012. Rapid tumour progression following 4 Cycles of gemcitabine + cisplatin from August 2012.

[0163] Commenced NUC-1031 on 12th February 2013 on 375 mg/m² twice weekly, and completed 2 Cycles. During Cycle 1 patient had symptomatic relief on treatment and a general improvement in mood and wellbeing. Developed transient (G3) transaminitis (ALT and AST). Lymphoedema, which was present at baseline, was progressing during Cycle 2.

[0164] Also developed a pleural effusion (G3) that was assessed as unlikely to be related to study drug.

Stable Disease to RECIST (3 months).

Patient 010 Endometrial Cancer: Stable Disease**Female (60 years)**

[0165] Diagnosed with endometrial cancer (stage IV, grade 3) in 2012. In February 2012 the patient received radiotherapy to a pelvic mass and 6 Cycles of carboplatin + paclitaxel. On disease progression in November 2012 received 3 Cycles of paclitaxel, and then rapidly progressed through a Cycle of Megace in January 2013.

[0166] Commenced NUC-1031 on 19th March 2013 on 750 mg/m² weekly, and completed 1 Cycle. Developed neutropenia (G3) during Cycle 1, which resolved after a few days, following intervention with GCSF. Dose reduced to 500 mg/m² for Cycle 2 and completed 5 Cycles at this dose. Patient had transient Grade 3 anaemia. Reduction in CA125 from 727 to 488. Patient had Stable Disease on study and this was maintained for a further 3 months after stopping NUC-1031.

Stable Disease to RECIST (9 months).

Patient 011 Uterine Carcinosarcoma: Stable Disease**Female (67 years)**

[0167] Diagnosed with uterine carcinosarcoma (recurrent MMMT of the uterus) and liver, lung and para-aortic nodal metastases. Surgery was performed in June 2011 with a radical hysterectomy and bilateral salpingo-oophorectomy. From June 2011 to November 2012 the patient received adjuvant cisplatin and doxorubicin - 6 cycles completed (good response to treatment with almost complete remission).□

June 2012 underwent further surgery with an anterior exenteration, dissection of rectum and repair and formation of ileal conduit with end-to-end anastomosis. Further surgery in June 2012 when she underwent a laparotomy with implantation of a ureter in to her ileal conduit and a Hartman's procedure for a rectal fistula. March 2013 completed 6 Cycles of weekly paclitaxel but with tumour progression through this course of treatment.

[0168] Commenced NUC-1031 on 15th April 2013 on 375 mg/m² twice weekly, and completed 2.6 Cycles. Patient had stable disease to RECIST with a reduction in tumour volume of 26%. During treatment had transient neutropaenia (G3) and a low haemoglobin (G2). At patient's request, changed to weekly schedule at 625 mg/m² for Cycle 4, and completed 1 further Cycle. The end of Cycle 4 (end Month 4) CT scan in August 2013 showed progressive disease. Three new lesions appeared: 2 in the lung, and 1 retro cava lymph node. The original lesion in the liver had continued to decrease in size and the other target lesion in the lung had remained stable. The patient was withdrawn from the study.

Stable Disease to RECIST (4 months).

Patient 012 Cholangiocarcinoma: Stable Disease

Female (48 years)

[0169] Diagnosed with Stage IV, Grade 3 cholangiocarcinoma in 2013 following investigation for right-sided abdominal pain. CT scan showed liver, lung and peritoneal metastases. From January - April 2013 the patient received 3 Cycles of cisplatin + gemcitabine, with rapid disease progression.

[0170] Commenced NUC-1031 on 16th May 2013 on 375 mg/m² twice weekly, and completed 3 Cycles. At patient's request changed to weekly schedule at 625 mg/m² for Cycle 4, completed 3 further Cycles and tolerated treatment well. Patient had Stable Disease at end of study and had a further two Cycles, with a total of 8 Cycles.

Stable Disease to RECIST (8 months).

Patient 013 Cervical Cancer: Partial Response

Female (51 years)

[0171] Diagnosed with inoperable, poorly differentiated squamous cell cervical cancer (Stage 2b, G2/3) in September 2011. She was treated with cisplatin (4 Cycles) plus radiotherapy and was said by the referring clinician to have a "good response".

[0172] In July 2012 the patient developed disease progression and, between July 2012 and November 2012, was given 6 doses of carboplatin + paclitaxel plus Cediranib (CIRCCA trial) and achieved stable disease. In April 2013 MRI scan showed disease progression with increased iliac lymph node involvement.

[0173] Commenced on NUC-1031 on 28th May 2013 on 750 mg/m² weekly, and completed 2 Cycles. Dose reduced to 625 mg/m² and completed 4 further Cycles. Had lower pelvic pain prior to study but had relief of this pain on treatment, with significant reduction of opioid usage. The patient had problems with recurrent urinary tract infections both before starting NUC-1031 and during treatment because of bilateral metal ureteric stents.

[0174] Patient had a Partial Response at completion of the ProGem1 study and then completed a further 3 Cycles on a reduced dose of 500 mg/m² weekly, with a total of 9 Cycles of NUC-1031. Patient's tumour has shrunk to the extent that she was being re-evaluated for further debulking surgery.

Partial Response to RECIST (9 months, PFS 11 months).

Patient 014 Mesothelioma: Progressive Disease

Female (51 years)

[0175] Patient diagnosed with a recurrent epithelioid mesothelioma in the right hemi-thorax in 2012. Received 4 Cycles of permutrexed + carboplatin. On progression in December 2012 entered a clinical study to receive dasatinib but was unresponsive and had disease progression. Commenced on NUC-1031 on 20th June 2013 on 750 mg/m² weekly, and completed 1 Cycle. Dose reduced to 625 mg/m² and completed 1 further Cycle. Withdrawn from study. Progressive Disease.

Patient 015 Cancer (Unknown Primary): Partial Response

Male (54 years)

[0176] Diagnosed with cancer of unknown primary in September 2012, having been investigated for symptoms of abdominal pain.

[0177] He was noted at that time to have liver and lung metastases. A liver biopsy showed a poorly differentiated carcinoma with focal glandular differentiation. Received 8 Cycles of epirubicin + cisplatin + capecitabine from October 2012 until April 2013 within the "CUP" clinical study but developed Progressive Disease with oedema, pleural effusion and ascites, with a 20 kg weight gain. Patient experienced severe nausea and vomiting and fatigue on this regimen.

[0178] Commenced on NUC-1031 on 20th June 2013 on 750 mg/m² weekly, and received 2 doses. Dose reduced to 625 mg/m² to complete Cycle 1 and received 5 further Cycles at the lower dose. On study entry patient had marked lower limb oedema and abdominal ascites (approximately 20 Kg). Following Cycle 1 it was reported that the oedema and ascites had gone and the patient was feeling much better. During Cycle 1 Day 8 developed thrombocytopenia (G3), lymphopenia (G3) and neutropenia (G2) which caused a two week treatment delay. End of Cycle 2 scan showed a reduction in all target lesions with a RECIST assessment of Stable Disease. End of Cycle 4 scan showed further reduction in all target lesions, with a RECIST assessment of a Partial Response, which was sustained until end of study. Requested compassionate continuation and completed 3 further Cycles at this dose. Was beginning to show a consistent drop in blood counts following Day 8 of each Cycle. From Cycle 10 dose reduced further to 500 mg/m² with the desired effect and completed a further 10 Cycles (19 in total).

[0179] Most recent CT scan on 9th January 2015 showed sustained Partial Response (approximatey 58% reduction in tumour size) and the target mesenteric node no longer visible. Had fluid build up in both legs during Cycle 10 but responded well to spironolactone and completely resolved. Replaced Hickman line on 12th December 2014 following 19 months in situ, with no adverse effect. Patient remains clinically very well. Following discussions with CI Patient is happy to have a treatment break and will be referred back to oncologist to discuss options.

Partial Response to RECIST (20+ months; PFS 24+ months) ongoing.

Patient 017 Lung Cancer: Partial Response

Female (60 years)

[0180] Diagnosed with lung adenocarcinoma, with lung, liver and adrenal metastases in September 2011. Also had disease in mediastinal, intra abdominal and cervical lymph nodes. The patient had a pleurodesis performed in December 2011 followed by 3 Cycles of cisplatin + pemetrexed which she completed in March 2012 and achieved a partial response. From March

- September 2012 she received a total of 6 Cycles of docetaxel, with a partial response. In April 2013 she had disease progression and was re-challenged with docetaxel, but progressed through 2 Cycles of docetaxel.

[0181] Commenced on NUC-1031 in July 2013 on 750 mg/m² weekly and received 1 dose. Dose reduced to 625 mg/m² and she received 2 doses to complete Cycle 1 and has completed 5 further Cycles. Had 4 weeks of treatment delays due to lung infections and low platelets. Significant response in diseased lymph nodes, particularly in the neck. Target lesions continued to shrink which was evident on post Cycle 2 and Cycle 4 CT scans. At this stage, RECIST assessment classified the response as Stable Disease.

[0182] At the end of Cycle 6 a CT scan showed further reduction and the RECIST assessment was changed to a Partial Response in all target lesions at end of study. Requested continuation on a compassionate use basis and completed 3 further Cycles. Patient tolerated NUC-1031 well, with improvement in hoarse voice and dysphagia. End of Cycle 9 scan on 17th April 2014 showed Progressive Disease with a growth on target lesions and new hepatic lesions. Withdrawn from study.

Partial Response to RECIST (3 months; PFS 10 months).

Patient 018 Lung Cancer: Stable Disease

Female (65 years)

[0183] Diagnosed with squamous cell lung cancer May 2011. Received gemcitabine + cisplatin, 6 Cycles from June to October 2011 within the SQUIRE trial. Disease relapse in April 2012 and she received single fraction palliative radiotherapy to right hilum and docetaxel 6 Cycles from May until September 2012.

[0184] Progressive disease October 2012. Commenced erlotinib December 2012 for 3 months but in March 2013 was found to have progressive disease in the right hilum.

[0185] Commenced on NUC-1031 on 25th July 2013 on 625 mg/m² weekly, and completed 4 Cycles. Stable disease to RECIST (4 months). Patient withdrawn from study due to symptomatic deterioration caused by vena caval obstruction. Received low dose radiotherapy in an attempt to resolve the obstruction and recommenced NUC-1031 on the compassionate access programme. Following one further dose of NUC-1031 it was agreed to withdraw patient from the compassionate access programme. The bulk of the patient's disease was stable on withdrawal.

Stable Disease to RECIST (4 months).

Patient 021 Fallopian Tube Cancer: Partial Response

Female (61 years)

[0186] Diagnosed with recurrent Stage 2a, Grade 2 endometrioid adenocarcinoma of the ovary in 2008. She received 6 Cycles of carboplatin + paclitaxel, completed October 2008. In June 2011 she relapsed and was noted to have pleural, subcapsular liver, omental and mesenteric tumour nodules and was recruited into the ICON6 study, receiving 6 Cycles of carboplatin plus paclitaxel +/- cediranib. She achieved a partial response to therapy. Remained on maintenance cediranib until March 2012 when treatment was discontinued due to rising CA125 and progressive peritoneal disease.

[0187] From March to July 2012 she received 6 Cycles of weekly paclitaxel with good radiological response initially of the peritoneal disease. □ In February 2013 she was found to have a new effusion and an increase in the peritoneal disease. She commenced carboplatin + paclitaxel and daily AKT inhibitor on the AKTRES study but with disease progression (new pelvic mass) in May 2013 after 3 Cycles.

[0188] Commenced on NUC-1031 on 28th August 2013 on 625 mg/m² weekly, and completed 6 Cycles. Noticed a significant reduction in abdominal ascites; required drainage every two weeks prior to coming on study and has not required further drainage since commencing NUC-1031. Patient tolerated NUC-1031 well. Stable disease to RECIST at end of study. Requested continuation on compassionate use basis and completed one further Cycle. End of Cycle 7 CT scan on the 26th February 2014 showed a further reduction in tumour volume which confirmed Partial Response to RECIST. Significant CA125 Response: 91% reduction from baseline (372) to end of Cycle 6 (35).

[0189] Best overall response to date is Partial Response (3 months) according to RECIST or Partial Response (9 months) according to GCIG criteria.

Partial Response.

Patient 024 Cancer of Unknown Primary: Progressive Disease**Female (51 years)**

[0190] Diagnosed with cancer of unknown primary in April 2012. Received CAPOX, 8 Cycles from April to October 2012. Received irinotecan in October 2012 with addition of bevacizumab in November 2012, but without response.

[0191] Commenced on NUC-1031 on 26th September 2013 on 675 mg/m² weekly, and completed 2 Cycles. End of Cycle 2 CT scan showed progressive disease.
Progressive Disease.

Patient 025 Mesothelioma: Stable Disease

Male (54 years)

[0192] Diagnosed with T4 N3 M0 epithelioid mesothelioma of the right lung in March 2013. Received 4 Cycles of pemetrexed + cisplatin from May to August 2013.

[0193] Commenced on NUC-1031 on 23rd October 2013 on 725 mg/m² weekly, and completed 4 Cycles. End of C4 CT Scan showed Progressive Disease. Withdrawn from study. Stable Disease to RECIST (4 months).

Patient 026 Colorectal Cancer: Stable Disease

Female (63 years)

[0194] Diagnosed with colorectal cancer, T4 N2, with lung and bladder metastases in February 2007. Received adjuvant FOLFOX, 12 Cycles, November 2007. Developed pelvic recurrent disease and received capecitabine 2009.

[0195] On relapse in 2012 received FOLFIRI in September 2012 and capecitabine + irinotecan until January 2013. In July 2013 CT showed Progressive Disease, presacral tumour recurrence causing destruction of sacrum, and a lung nodule.

[0196] Commenced on NUC-1031 on 17th October 2013 on 725 mg/m² weekly, and completed 4 Cycles. Significant improvement in pain, with dramatic reduction in use of opioid analgesia. End of Cycle 4 CT Scan showed Progressive Disease.
Stable Disease to RECIST (4 months).

Patient 027 Ovarian Cancer: Stable Disease

Female (46 years)

[0197] Diagnosed with serous adenocarcinoma of both ovaries in December 2009. Following total hysterectomy, bilateral salpingo-oophorectomy and omentectomy she received 6 Cycles carboplatin + paclitaxel and achieved a Complete Response in May 2010. The patient relapsed in June 2011 and received carboplatin + paclitaxel 6 Cycles (ICON6 Study). In December 2012 the patient was given a further 3 Cycles of gemcitabine + carboplatin but had an allergic reaction to carboplatin which was switched to cisplatin. She completed 6 Cycles in total and achieved a partial response in April 2013. This was followed by 6 months of tamoxifen but in July 2013 a CT scan showed new mediastinal lymph node involvement and the CA125 levels increased. A CT scan in October 2013 showed an increase in the size of peritoneal deposits.

[0198] Commenced on NUC-1031 on 30th October 2013 on 725 mg/m² weekly. Developed elevated ALT (G3) following Cycle 1 Day 1, raised from 96 at baseline to 256 on day 7, a DLT for this cohort. ALT recovered to G2 a few days later to allow patient to receive Cycle 1 Day 8 at the reduced dose of 675 mg/m². Completed Cycle 1 at reduced dose and went on to receive a further 3 Cycles. Patient achieved Stable Disease to RECIST with a reduction in tumour volume of 23%. CA125 has reduced from 188 at baseline to 99 at end of Cycle 6. Dose was further reduced for Cycle 5 to 625 mg/m² due to mild neutropenia. Completed study at this dose with no further issues. Requested compassionate continuation and received 1 further Cycle.

Stable Disease to RECIST (8 months).

Patient 029 Breast Cancer: Stable Disease

Female (53 years)

[0199] Diagnosed with metastatic breast cancer (ER and PGR positive), with multiple bone and hepatic metastases in 2002. Received 6 Cycles of FEC, adjuvant radiotherapy and tamoxifen with goserelin. In 2010 new bone metastases detected and treated with Zoladex, letrozole and pamidronate. July 2011, switched to Zoladex and exemestane, which was augmented with Faslodex in November. On further progression in 2012 received capecitabine + Zometa, followed by paclitaxel for 3 Cycles only. Commenced treatment with rucaparib in May 2013. Progressive hepatic disease in July 2013 and received gemcitabine + carboplatin for 3 Cycles.

[0200] Commenced on NUC-1031 on 14th November 2013 on 725 mg/m² weekly. Completed 3 Cycles. Unfortunately suffered a fatal cardiac arrest while at home, not study related.
Stable Disease to RECIST (4 months).

Patient 030 Ovarian Cancer: Stable Disease

Female (62 years)

[0201] Diagnosed with serous adenocarcinoma of the ovary in 2012. Received adjuvant carboplatin + paclitaxel for 6 Cycles to July 2012, achieved complete response. Progressive disease in August 2013, commenced carboplatin + caelyx, progressed following 3 Cycles.

[0202] Commenced on NUC-1031 on 21st November 2013 on 725 mg/m² weekly. Completed 3 Cycles and tolerated study drug well. End of Cycle 2 CT scan showed Stable Disease to RECIST. Unstable dietary issues resulting in dehydration and malnutrition, which led to lengthy treatment delays. Withdrawn from study.

Stable Disease to RECIST (3 months).

Patient 031 Cholangiocarcinoma: Progressive Disease**Female (76 years)**

[0203] Diagnosed with cholangiocarcinoma in July 2013. On the 27th July she underwent a modified Whipple's procedure. At the time of surgery she was noted to have multiple liver metastases. In August 2013 she commenced gemcitabine + oxaliplatin which was given every two weeks for 6 Cycles.

[0204] Commenced on NUC-1031 on 9th December 2013 on 750 mg/m² weekly and completed 2 Cycles. End of Cycle 2 CT scan showed Progressive Disease. Withdrawn from study.

Patient 032 Oesophageal Cancer: Stable Disease**Male (56 years)**

[0205] Diagnosed with squamous cell carcinoma of the oesophagus in June 2013. Received 3 Cycles of cisplatin + capecitabine from July to September 2013. Progressive disease with peritoneal and lung metastases. Oesophageal stent inserted in October 2013 to control symptoms of dysphagia.

[0206] Commenced on NUC-1031 on 16th December 2013 on 750 mg/m² weekly and

completed 2 Cycles. End of Cycle 2 CT scan showed Stable Disease to RECIST. Patient was having difficulties with a lung/trachea fistulae. Withdrawn from study due to clinical progression. Stable Disease to RECIST (2 months).

Patient 033 Cholangiocarcinoma: Stable Disease

Female (37 years)

[0207] Diagnosed with advanced cholangiocarcinoma in June 2013 with liver, peritoneal and para aortic lymph node metastases and small pulmonary nodules. In June she had a liver biopsy which showed a probable poorly differentiated cholangiocarcinoma, with some features to suggest a liver primary. In July 2013 she commenced chemotherapy with gemcitabine and cisplatin and received 6 Cycles omitting some of Cycle 5 due to an admission for neutropenic sepsis.

Unfortunately, although her interval scan showed a partial response her post treatment CT scan on the 28th November 2013 showed progressive disease with a stable liver lesion but an increase in the size of her pulmonary metastases and some new peritoneal deposits. She also is known to have a lytic sternal lesion.

[0208] Commenced on NUC-1031 on 3rd January 2014 on 750 mg/m² weekly and completed 4 Cycles. End of Cycle 2 CT scan showed Stable Disease to RECIST. End of Cycle 4 scan showed progressive disease and patient was withdrawn from the study.

Stable Disease to RECIST (3 months)

Patient 036 Renal Carcinoma: Stable Disease

Male (20 years)

[0209] Diagnosed with medullary cell renal carcinoma in December 2012. Received 5 Cycles of gemcitabine + paclitaxel + carboplatin from January until July 2013 resulting in Stable Disease during treatment. Patient had treatment delays due to thrombocytopenia and neutropenia which required intervention with G-CSF. Following relapse in July 2013 commenced gemcitabine + doxorubicin but progressed through 2 Cycles in September 2013.

[0210] Commenced on NUC-1031 28th January 2014 on 825 mg/m² weekly and completed 4 Cycles. Patient experienced fatigue and tiredness during Cycle 1. Lorazepam was discontinued and he became much more alert. He reported that he was tolerating NUC-1031 much better than his previous regimen. End of Cycle 4 CT scan showed sustained Stable Disease with a

reduction in tumour volume of 7%. Following Cycle 5 Day 1 developed thrombocytopenia (G3) and dose was reduced to 750 mg/m². Following Cycle 5 Day 8 developed fatigue, loss of appetite and did not present for any further treatment. Withdrawn from study due to clinical progression.

Stable Disease to RECIST (5 months).

Patient 037 Pancreatic Cancer: Partial Response

Female (70 years)

[0211] Diagnosed with pancreatic adenocarcinoma in March 2013. Whipple's procedure was planned for 26th of March 2013 but due to extensive adhesions the cancer was non-resectable, but biopsies were taken. Liver wedge resection confirmed metastatic disease. Histology showed moderately differentiated adenocarcinoma. Patient received 6 Cycles of gemcitabine from May to October 2013. CT scan November 2013 suggested partial response of pancreatic tumour, but with new metastases in the lateral left lobe of the liver.

[0212] Commenced on NUC-1031 4th February 2014 on 1,000 mg/m² weekly and received 1 Cycle at this dose. At this time the DSMC decided to reduce the dose in all patients in this cohort to 900 mg/m² due to a DLT in one patient (patient 039). Patient received one further Cycle at the new dose. End of Cycle 2 CT scan showed Stable Disease to RECIST with an 18.4% reduction in tumour volume.

[0213] Pain in abdomen and back had significantly improved; patient was on oxycontin 80mg bd and had now stopped all morphine. Also had a very significant drop in tumour markers: CA19.9 from 15,000 at baseline to 4,000 and CEA from 536 at baseline to 42. Fatigue had become a major issue following each cycle. CT scan on 29th April 2014 showed a further reduction in tumour volume to 30% to achieve a Partial Response to RECIST. Patient had loss of appetite, severe fatigue and was withdrawn from the study.

Partial Response to RECIST (1 month; PFS 4 months).

Patient 038 Ovarian Cancer: Progressive Disease

Female (65 years)

[0214] Diagnosed with recurrent stage 3c grade 3 serous adenocarcinoma of the ovary in 2000. She underwent a total abdominal hysterectomy, with bilateral salpingo-oophorectomy and debulking surgery, leaving minimal residual disease, in November 2000. Received 6

Cycles of 3 weekly carboplatin + paclitaxel until March 2001. Following relapse in 2002 received 6 cycles of Carboplatin plus Etoposide to complete remission in March 2003. Further relapse in 2005 and had more debulking surgery, followed by carboplatin + gemcitabine x 6 Cycles which finished in July 2006 with complete response. Additional debulking surgery, including splenectomy, was required following relapse in 2009. This was followed by 6 Cycles of carboplatin + caelyx and a complete response was achieved. A right pelvic recurrence near the right external iliac vessel, which was considered to be inoperable, was noted in 2010. The patient received carboplatin and paclitaxel for 6 Cycles, which she completed in April 2011, with a partial response. In October 2011 she showed evidence of progression, received topotecan for 6 Cycles till March 2012 and had stable disease. At this time she underwent insertion of a right ureteric stent for hydronephrosis. In June 2012, after further disease progression with new bilateral lung metastases, she was started on weekly carboplatin + paclitaxol + bevacizumab to March 2013 followed by maintenance bevacizumab + letrozole to September 2013. This was followed by 3 Cycles of cyclophosphamide + bevacizumab, but interval scan showed progressive disease and on 11th November 2013 her treatment was discontinued. Right ureteric stent changed January 2014.

[0215] Commenced on NUC-1031 on 4th March 2014 on 900 mg/m² weekly and completed 2 Cycles. Main toxicity was delayed onset fatigue, which set in on days 3 to 4. End of Cycle 2 scan showed progressive disease with a 25% increase in tumour volume. Withdrawn from the study.

Progressive Disease.

Patient 040 Cholangiocarcinoma: Stable Disease

Female (69 years)

[0216] Diagnosed in May 2013 with intrahepatic grade 2 cholangiocarcinoma, with 11cm liver mass obstructing the common bile duct and causing jaundice. A biliary stent was inserted. Received 7 Cycles Gemcitabine + Cisplatin from August to December.

[0217] Commenced on NUC-1031 on 20th February 2014 on 1,000 mg/m² weekly and received 1 dose. Presented for Cycle 1 Day 8 on 27th February with fever, rigors and an elevated bilirubin. Was admitted, and source of infection (G3) was a stent blocked with tumour and a biliary tract cyst. Two new stents were working well. Cycle 1 was completed at 900 mg/m² due to DLT in that cohort. Completed 3 Cycles. End of Cycle 2 CT scan on 23rd May showed Stable Disease to RECIST with slight reduction in tumour volume from 85 at base to 82.1. CA 19.9 dropped from 664 at baseline to 155 on 4th June. Was admitted with delirium in June 2014 and diagnosed with a urinary tract infection. Further investigation also revealed progressive disease in the liver. Withdrawn from study.

Stable Disease to RECIST (4 months).

Patient 041 Breast Cancer: Stable Disease

Female (54 years)

[0218] Diagnosed with metastatic invasive ductal breast cancer (ER and PR +ve) with bilateral axillary nodes, lung and liver metastases. Received FEC x 3 Cycles, paclitaxel x 9 Cycles, capecitabine x 8 Cycles, euribulin x 3 Cycles and gemcitabine + carboplatin x 1 dose. Her last dose of chemotherapy was on the 19th of December 2013 and the last CT scan before enrolment showed progressive disease.

[0219] Commenced on NUC-1031 on 18th March 2014 on 900 mg/m² weekly and completed 3 Cycles. Had 2 treatment delays following Cycle 1 Day 8 and Day 15 due to neutropenia, which resolved spontaneously within one week. End of Cycle 2 CT scan showed Stable Disease to RECIST. CA 15.3 tumour marker was 726 at base 845 at C2 and was 824 on 19th May 2014. (This tumour marker has always been a reliable indicator of response in the past). Experiencing fatigue (G3) during Cycle 3 but had managed to reduce opioids significantly. NUC-1031 dose reduced to 825 mg/m² for Cycle 4. End of Cycle 4 scan showed progressive disease with increase at target sites and new bone lesions. Withdrawn from study.

Stable Disease to RECIST (4 months)

Patient 042 Cholangiocarcinoma: Progressive Disease

Male (48 years)

[0220] Diagnosed with metastatic cholangiocarcinoma in January 2013. Partial hepatectomy in February 2013. Commenced on the BILCAP trial observation arm (comparing capecitabine with observation after surgery for biliary tract cancer). On progression commenced on gemcitabine and cisplatin x 6 Cycles. On further progression in November 2013 he commenced capecitabine. However, this was stopped after two months as he developed angina. On further progression he was commenced on 5FU x 6 weeks but had progressive disease with lung, liver and bone metastases.

[0221] Last treatment was in January 2014. Received palliative radiotherapy for pain in right shoulder bone metastasis on the 20th of January 2014.

[0222] Commenced on NUC-1031 on 18th March 2014 on 900 mg/m² weekly and completed 2 Cycles. Experienced delayed onset fatigue (G2) on days 3-5 following study drug. End of Cycle 2 CT scan showed a 9% reduction in tumour volume of primary target lesion but showed new pulmonary and bone lesions. Withdrawn from the study. Progressive Disease.

Patient 043 Ovarian Cancer: Stable Disease

Female (54 years)

[0223] Diagnosed with stage 4, Grade 3 papillary serous peritoneal cancer. A total abdominal hysterectomy, with bilateral salpingo-oophorectomy and omentectomy performed in September 2007. Received carboplatin + paclitaxel x 4 Cycles followed by 2 Cycles of Carboplatin alone, due to neuropathy, and completed course in January 2008. In April 2011 underwent secondary debulking surgery for recurrent pelvic mass. Patient did not wish to have adjuvant chemotherapy or radiotherapy. Further recurrence of disease July 2012 and a stent inserted for hydronephrosis. Recurrent disease in August 2012 and commenced carboplatin + gemcitabine. CT scan in March 2013 revealed disease progression; patient completed 6 x Cycles of Caelyx in October 2013. Progressive disease early 2014 with pleural effusion, which required very regular drainage.

[0224] Commenced on NUC-1031 on 20th March 2014 on 900 mg/m² weekly and completed 6 Cycles. Received PET scan on 7th April 2014 which showed stable disease and SUV had gone down in some target tumours. CA125 had reduced from 1.099 at baseline to 783 at the beginning of Cycle 2. The volume of fluid from the pleural effusion also reducing (was draining 300ml per week, now 150 ml per week). Developed delayed onset fatigue (G2) on days 4 and 5 and G1 at all other times. End of Cycle 6 CT scan showed Stable Disease to RECIST with an overall 10% reduction in tumour volume from baseline. CA125 fell from 1,099 at baseline to 910 on 15th July. Completed study and requested compassionate continuation. Dose reduced to 750 mg/m² for Cycle 7 and received 1 further Cycle. Leg oedema developed to G3, no disease progression but new treatment options sought and withdrawn from study. Stable Disease to RECIST (13 months).

Patient 044 Lung Cancer: Stable Disease

Female (64 years)

[0225] Diagnosed with adenocarcinoma of the lung (left lower lobe) February 2010 following unresolved cough for 7 months. Between January 2011 - April 2012 patient received Gefitinib

250m, but had progressive disease. Received Afatinib from April 2012 to November 2012 (but dose reduction due to skin toxicity) but again with progressive disease. From November 2012 to June 2013 patient given Erlotinib after worsening cough and progression of the primary lesion. In June 2013 developed abnormal vision and black shadows in left eye and found to have choroidal metastases for which she received radiotherapy to both eyes, with improvement of her vision. In June 2013 - started pemetrexed + carboplatin for 6 Cycles followed by maintenance pemetrexed until 6th Feb 2014 when progressive disease was diagnosed.

[0226] Commenced on NUC-1031 on 27th March 2014 at 900 mg/m² weekly and had completed 2 Cycles. Had a G3 lung infection on 19th April which responded well to antibiotics. Commenced Cycle 2 on a reduced dose, 825 mg/m² and completed a further 2 Cycles. End of Cycle 2 CT scan showed Stable Disease to RECIST with a 10% reduction in tumour volume. Had 2 treatment delays for ascites drainage and two more for thrombocytopenia. End of Cycle 4 scan showed progressive disease in lung and new liver metastases. Withdrawn from study. Stable Disease to RECIST (5 months).

Patient 046 Adrenal Carcinoma: Stable Disease

Male (36 years)

[0227] Diagnosed in August 2011 with a large 20 x 19 x 9 cm adrenocortical carcinoma. Ki 67 35-40% mitotic count 25/50hpf, Weiss score 6 with tumour extending to 0.3 mm of external margins but no renal involvement. Received adjuvant mitotane until January 2012 when it was stopped due to nausea and diarrhoea. Recommened mitotane in June 2012. Relapsed in June 2013 with new liver metastases and commenced etoposide + carboplatin.

[0228] Staging CT scan on September 2013 showed differential response but overall stable disease, and he received a further 3 Cycles and remained stable on completion in November 2013. In January 2014 showed progressive disease in the liver.

[0229] Commenced on NUC-1031 on 16th April 2014 on 1,000 mg/m² weekly and completed 1 Cycle. During Cycle 1 experienced delayed onset fatigue, nausea and vomiting (all G3). Dose was reduced to 900 mg/m² for Cycle 2 and received a further 5 Cycles on this dose to complete the study. End of Cycle 6 CT scan showed a 12.6% reduction in tumour volume from baseline. Requested compassionate continuation and completed 3 further Cycles. Developed fatigue (G3) and neutropenia (G2) following Cycle 8 D1, was dose reduced to 750 mg/m² for Cycle 8 and received 2 further Cycles. Though improved to G1, fatigue continued, also developed nausea and vomiting and dose was further reduced to 625mg/m² for C11. Received 2 further doses. Withdrawn from study.

Stable Disease to RECIST (11 months).

Patient 048 Ovarian Cancer: Stable Disease

Female (63 years)

[0230] Diagnosed with stage 1a granulosa cell tumour of the ovary in 2000 and had total abdominal hysterectomy and bilateral salpingo-oophorectomy + omentectomy. On recurrence in February 2004 commenced 3 cycles of BEP (bleomycin + etoposide + cisplatin) to disease progression. Secondary debulking in July 2004.

[0231] In June 2006 underwent partial hepatectomy, splenectomy and excision of deposits from stomach and peritoneum. This was followed by radiofrequency ablation to liver deposits. Underwent laparotomy and resection of 4 further metastatic deposits in September 2008. In March 2009 commenced 3 weekly carboplatin + paclitaxel, with a mixed response. Changed to weekly carboplatin + low dose paclitaxel on May 12th 2009. Completed in October 2009 with PR and CA-125 negative. Further debulking surgery October 2011, with complications requiring prolonged stay in ITU. CT scan in April 2014 showed lesion in segment 8 of liver had increased in size, new small peritoneal metastases in the gastro-hepatic ligament and some new small volume lymphadenopathy in the small bowel mesentery and further small peritoneal deposits in the pelvis around the recto-sigmoid junction.

[0232] Commenced on NUC-1031 on 29th April 2014 on 1,000 mg/m² weekly and received 1 dose. Following Cycle 1 Day 1 developed; ALT, (G3, a DLT); AST and neutropaenia (G2); ALP (G1). Dose reduced to 900 mg/m² for Cycle 1 Day 8. ALT returned to G3 following days 8 and 15. Dose for Cycle 2 reduced to 825 mg/m². Results from PET scan at end of Cycle 1 showed stable disease and reduction in SUV at target sites. In January 2014 inhibin B was 430 and increased to 1,038 prior to study entry. July 2014 inhibin B had stabilized to 1,106, August 1148, September 1053. Dose reduced for Cycle 4 Day 15 to 750 mg/m² due to neutropenia (G3) despite intervention with G-CSF. Completed study on this dose. End of Cycle 6 CT scan on 11th November confirmed Stable Disease to RECIST. Requested compassionate continuation. Due to diarrhoea following each dose, commenced Cycle 7 at the reduced dose of 625 mg/m². Developed emboli close to Hickman line, and although this responded to clexane the patient was withdrawn from study.

Stable Disease to RECIST (8 months).

Patient 050 (202) Oesophageal Cancer: Progressive Disease

Female (41 years)

[0233] Diagnosed with Stage 4 squamous cell carcinoma of the oesophagus with liver metastases in November 2013. Received 6 Cycles of cisplatin + capecitabine from December 2013 until April 2014. Her post-treatment scan showed progressive disease, and new lung deposits.

[0234] Commenced on NUC-1031 on 21st May 2014 on 900 mg/m² weekly and received 3 Cycles. Following Cycle 1 Day 1 developed ALT and fatigue, both G3, and had dose reduction for Cycle 1 Day 8. End of Cycle 2 scan seemed to show Disease Progression, though uncertainty over some baseline lesions.

[0235] As patient was deriving clinical benefit with improvement in her dysphagia, it was decided to allow one more cycle. End of Cycle 3 scan confirmed Disease progression and patient was withdrawn from study.

Progressive Disease.

Patient 051 (203) Anal Cancer: Progressive Disease

Female (51 years)

[0236] Diagnosed with metastatic squamous cell carcinoma of the anus in October 2013. Progressed following 6 Cycles of cisplatin + 5FU from October 2013 until March 2014.

[0237] Commenced on NUC-1031 on 3rd June 2014 on 900 mg/m² weekly and has received 2 Cycles. Required blood transfusion during Cycle 2 but tolerated treatment well. End of Cycle 2 CT scan showed progressive disease.

Progressive Disease.

Patient 052 (204) Oesophageal Cancer: Stable Disease

Male (66 years)

[0238] Diagnosed with stage IV oesophageal cancer in December 2012. Received FOX from January 2013 until July 2013, with a Partial Response. Showed Progressive Disease in April 2014. Oesophageal stent inserted.

[0239] Commenced on NUC-1031 on 10th June 2014 on 900 mg/m² weekly and completed 1 Cycle. Due to fatigue dose was reduced to 825 mg/m² for Cycle 2, with good effect. Completed 2 further Cycles at this dose. End of Cycle 2 CT scan showed Stable Disease to RECIST. Continues to have bone pain but scans revealed that this is not disease related. Dysphagia was becoming exacerbated. End of Cycle 4 CT scan on 10th November revealed Disease Progression.

Stable Disease to RECIST (5 months).

Patient 053 (205) Colon Cancer: Progressive Disease

Female (31 years)

[0240] Diagnosed with a T3 N1 M0 adenocarcinoma of the colon in 2008. Resected and received 12 Cycles of FOLFOX, which was completed in 2009. On progression in 2011, received 13 cycles of FOLFIRI (6 of the cycles included Avastin). With further progression in 2012, received 12 Cycles of FOLFOX (6 of the Cycles included Avastin). Further Progressive Disease in January 2014, with metastases to the lungs and vertebrae. Received radiotherapy to the spine.

[0241] Commenced on NUC-1031 on 12th June 2014 on 900 mg/m² weekly and has completed 1 Cycle. Has developed a series of infections, and has required a de-functioning ileostomy. Following many treatment delays received Cycle 2 Day 15 and end of Cycle 2 CT scan showed Progressive Disease with new lesions in the lung and liver.

Progressive Disease.

Patient 055 (207) Ovarian Cancer: Stable Disease

Female (42 years)

[0242] Diagnosed in January 2002 with stage 2c grade 1 papillary serous adenocarcinoma of the ovary. Underwent, total abdominal hysterectomy, bilateral salpingo-oophorectomy and omentectomy with 6 Cycles of adjuvant carboplatin + paclitaxel, completing treatment in June 2002.

[0243] In January 2012 developed a new grade 3 stage 3c primary peritoneal cancer involving the recto-sigmoid junction. Underwent posterior exenteration, comprising resection of caecum, rectum and sigmoid plus omentectomy and peritoneal stripping. From March to August 2012 received 6 Cycles of adjuvant carboplatin + paclitaxel.

[0244] On disease recurrence in January 2013 received 6 Cycles of weekly paclitaxel until May 2013. Following further progression in October 2013 commenced 3 weekly paclitaxel + carboplatin + daily AKT inhibitor within the AKTRES study. In May 2014 CT showed new lesions and progressive disease.

[0245] Commenced on NUC-1031 on 2nd July 2014 on 900 mg/m² weekly and received 1 Cycle. Dose was reduced to 825 mg/m² for Cycle 2 due to fatigue and completed 4 further Cycles. Experienced nausea and vomiting post chemotherapy, dose reduced to 625 mg/m² for Cycle 6 and completed one further cycle. End of Cycle 6 CT scan on 16th December 2014 showed continued Stable Disease to RECIST with a reduction in tumour volume of 18% from baseline. Patients CA 125 was 36 at baseline and was 24 in January 2015. Completed study and requested compassionate continuation. Received 2 further Cycles under the Compassionate Access Programme with no further issues. Patient elected to come off study as she was traveling a great distance and requested the break.
Stable Disease to RECIST (10+ months).

Patient 057 (209) Ovarian Cancer: Stable Disease

Female (58 years)

[0246] Diagnosed with Stage 3c grade 3 serous ovarian cancer in October 2011. Received 3 Cycles of neo-adjuvant carboplatin + taxol, but developed taxol allergy on Cycle 3. Underwent posterior exenteration, ovarian debulking, anterior resection with a primary anastomosis, and omentectomy on 21st December 2011. Completed 3 Cycles of carboplatin + docetaxel in March 2012. Relapsed in June 2013 and received 6 Cycles of carboplatin + caelyx until November 2013. In February 2014 had CT evidence of recurrent disease and received 3 Cycles of carboplatin + gemcitabine from March till June 2014.

[0247] Commenced on NUC-1031 on 9th July 2014 on 900 mg/m² weekly and received 2 doses. Presented for Cycle 1 Day 15 on 30th July with anaemia and ascites both G3. Dose reduced to 825 for Cycle 2 and received 2 further Cycles. End of Cycle 4 CT scan showed continued Stable Disease to RECIST with a reduction in tumour volume of 10% from baseline.
Has a pleurx drain in and approximately 1,000 mls of very bloody fluid are withdrawn every other week. Further dose reduction to 750 mg/m² from Cycle 4 Day 1 due to fatigue, received one further cycle. Abdominal fluid increasing. Withdrawn from study due to clinical progression.
Stable Disease to RECIST (5 months)

Patient 058 (210) Lung Cancer: Stable Disease

Male (54 years)

[0248] Diagnosed with metastatic non-small cell lung adenocarcinoma, with lymph node and bone metastases in January 2014.

[0249] Commenced 3 Cycles of cisplatin and pemetrexed with a response of Stable Disease. March 2014 commenced maintenance pemetrexed, received 4 Cycles until April 2014. In May 2014 showed progressive disease with bilateral pulmonary metastases and received 1 more dose of pemetrexed.

[0250] Commenced on NUC-1031 on 14th July 2014 on 825 mg/m² weekly and received 1 Cycle. Cycle 1 Day 15 was delayed one week due to G3 transaminitis (ALT), a DLT, and dose was reduced to 750 mg/m². However some of the elevation in liver enzymes may be due to a congenital liver condition. Received 1 further Cycle at 750mg/m². End of Cycle 4 CT scan showed Progressive Disease with new lesions in the bones.

Stable Disease to RECIST (4 months)

Patient 059 (211) Cervical Cancer: Stable Disease**Female (52 years)**

[0251] Diagnosed with stage IV squamous cell carcinoma of cervix in December 2012. Commenced on carboplatin + taxol chemotherapy, which was stopped due to toxicity, especially from the taxol, (rash and itching), in February 2013. Commenced 6 Cycles of cisplatin + topotecan from March 2013 until July 2013 and achieved Stable Disease. CT scan in October 2013 showed disease progression. She received 30Gy in 10 fractions pelvic radiotherapy, which was completed in November 2013. Commenced gemcitabine + carboplatin in December 2013 and received 3 Cycles. However, an interval CT scan in February 2014 showed disease progression.

[0252] Commenced on NUC-1031 on 24th July 2014 on 825 mg/m² weekly and completed 1 Cycle. Cycle 1 Day 15 was delayed for 1 week due to thrombocytopenia (G3). Dose reduced to 750 mg/m² for Cycle 2 and completed 2 further Cycles. End of Cycle 4 CT scan showed continuing Stable Disease to RECIST. Dose reduced to 625 mg/m² for Cycle 4 due to fatigue (G3) experienced during Cycle 3 with good effect. Completed 2 further Cycles at this dose. Urinary stents made patient very uncomfortable and replaced in January 2015. During Cycle 6

patient reported to be very tired and elected to come off study.
Stable Disease to RECIST (7 months).

Patient 060 (212) Pancreatic Cancer: Progressive Disease

Male (83 years)

[0253] Diagnosed with metastatic pancreatic cancer (moderately differentiated adenocarcinoma), with multiple liver metastases, in January 2014. Elected to have palliative chemotherapy on the Maestro study (gemcitabine on day 1, 8 and 15 and hypoxia activated TH302) from January to June 2014 but had progressive disease.

[0254] Commenced on NUC-1031 on 5th August 2014 on 825 mg/m² weekly and completed 1 Cycle. Presented on 10th September with ALP, AST, ALT, all G3. Required new stent. Following treatment delays completed Cycle 2 on 1st October. CT scan showed Progressive Disease with new liver lesions.

Progressive Disease.

Patient 061 (213) Colorectal Cancer: Stable Disease

Female (53 years)

[0255] Diagnosed with colon cancer in 2012. Surgery, involving bilateral salpingo-oophorectomy, omentectomy and a loop ileostomy. Commenced 12 Cycles of FOLFOX from January to July 2013. Following disease recurrence in April 2014 she received 8 Cycles of FOLFIRI and cetuximab from April to July 2014. Had severe nausea and vomiting to all chemotherapy.

[0256] Commenced on NUC-1031 on 11th August 2014 on 825 mg/m² weekly and completed 4 Cycles. Had no nausea or vomiting during treatment. End of Cycle 2 CT scan showed Stable Disease to RECIST with a 2% reduction in tumour volume. End of Cycle 4 CT scan showed Progressive Disease with new lesions in the spleen and liver.

Stable Disease to RECIST (3 months).

Patient 063 (215) Ovarian Cancer: Stable Disease

Female (78 years)

[0257] In May 2011 was diagnosed with concurrent vulval melanoma (Clark's level 4) and a stage 3b ovarian cancer. Commenced 3 Cycles of carboplatin + paclitaxel from August to September 2011, followed by interval de-bulking surgery, comprising total abdominal hysterectomy, bilateral salpingo-oophorectomy and omentectomy. She then received a further 3 Cycles of carboplatin + paclitaxel, completing treatment in November 2011. On disease progression, commenced 6 Cycles of gemcitabine + carboplatin + Avastin from September 2013 to February 2014. Then received 2 Cycles of caelix from April to June 2014.

[0258] Commenced on NUC-1031 on 27th August 2014 on 825 mg/m² weekly and received 1 Cycle. Dose reduced to 750 mg/m² for Cycle 2 Day 15 due to anaemia and neutropenia and received 2 further Cycles. End of Cycle 2 CT scan showed Stable Disease to RECIST. Dose reduced to 625 mg/m² for Cycle 4 Day 1 due to fatigue (G3), experienced on Cycle 3. Developed persistent shortness of breath. Removed from study. Stable Disease to RECIST (3 months).

Patient 064 (216) Trophoblastic Cancer: Progressive Disease

Female (38 years)

[0259] In June 2011 was diagnosed with recurrent stage 3 mixed placental site and epithelioid trophoblastic tumour (PSTT/ETT). Following radical hysterectomy and lymph node sampling received adjuvant chemotherapy with paclitaxel + cisplatin ultimately with paclitaxel + etoposide from June to October 2011 followed by surgery for lymph leakage in November 2011 and a bilateral ureteric implantation February 2012.

[0260] In February 2013 underwent a left oophorectomy and resection of bladder serosa for recurrent disease and was given adjuvant chemotherapy of high dose etoposide from March to July 2013, followed by autologous stem cell transplant. January 2014 underwent posterior exenteration, comprising resection of the upper vagina, rectum and bladder, removal of the left ovary, anterior vagina, a mesenteric nodule and right ureter. Received 5 Cycles of pemetrexed + carboplatin from February to May 2014. This was switched to gemcitabine + carboplatin, due to rising HCG levels. Received 2 Cycles but interval CT scan showed left lower lobe lung lesion which was considered inoperable.

[0261] Commenced on NUC-1031 on 3rd September 2014 on 825 mg/m² weekly and has received 2 Cycles. End of Cycle 2 CT scan showed Progressive Disease, growth in existing lesions in lungs and peritoneum.

Progressive Disease.

Patient 066 (218) Colorectal Cancer: Stable Disease**Male (65 years)**

[0262] In May 2011 was first diagnosed with pT4b pN0 moderately differentiated adenocarcinoma of the sigmoid colon. Underwent an anterior resection in November 2011 and commenced FOLFOX chemotherapy in January 2012. Developed peripheral neuropathy following 3 Cycles and was switched to 5FU monotherapy. Also troubled with delays due to diarrhoea and malaise. Following 3 months on 5FU showed a stable marker response but a mixed response on CT scan in July 2012. Remained stable off treatment until May 2013 showed progressing on his CT scan with the tumour markers levels doubling. Re-commenced 5FU + Avastin, which was completed in October 2013. Treatment was complicated with numerous hospital admissions with chest infections and chest pain. Received 8 Cycles of cetuximab from March to July 2014. CT scan showed disease progression and switched to 5FU plus Avastin. In June 2014 underwent a laparotomy and adhesiolysis because of adhesions from metastatic deposits within his peritoneum. Received a further cycle of Avastin but, with a rising CEA, was discontinued. Has many co morbidities, COPD, ischemic heart disease, and congestive cardiac failure.

[0263] Commenced on NUC-1031 on 16th September 2014 on 825 mg/m² weekly and received 2 Cycles. Dose reduction to 750 mg/m² for Cycle 3 due to fatigue and received 1 further Cycle. End of Cycle 2 CT scan showed Stable Disease to RECIST. Had two recent admissions for complications from a hernia, which resulted in treatment delays. Unscheduled CT scan on 30th December showed continued Stable Disease with reduction in tumour volume of 16% from baseline. Dose further reduced to 625 mg/m² for Cycle 4 due to fatigue. Has received 1 further Cycle at this dose with no further issues.

[0264] Admitted during Cycle 5 with acute back pain, old fracture noted (not study related). Continues under surgical evaluation. Withdrawn from study due to treatment delays. Stable Disease to RECIST (8+ months).

Patient 067 (219) Osteosarcoma: Stable Disease**Male (38 years)**

[0265] In May 2011 was diagnosed 24 Feb 2012 with osteosarcoma of proximal right tibia. Received 6 Cycles of cisplatin + doxorubicin + methotrexate from February to November 2012.

Had a proximal tibial replacement on November 2012 and received post chemotherapy mifamurtide for 6 months. CT scan in August 2014 showed metastatic recurrence with new intrapulmonary and pericardial lesion, inferior to IVC and adjacent to right atrium.

[0266] Commenced on NUC-1031 on 30th September 2014 on 825 mg/m² weekly and has received 5 Cycles. End of Cycle 4 CT scan showed Stable Disease to RECIST. Tends to develop neutropenia G2 towards the end of each Cycle but counts bounce back quickly. Completed study on 3rd March 2015. Tolerated study drug well. EOS CT scan showed Stable Disease to RECIST with an increase in 1% from baseline. Scan also showed significant calcification to tumour. Patient requested compassionate continuation of study drug and will completed C7 on 2nd April 2015 at the reduced dose of 750mg/m². Assessed by the thoracic surgeon who will operate on the left lung to remove the target lesion on the lower lobe on 26th April 2015. Thoracic surgeons removed the calcified target lesion in the lower lobe on 26th April 2015. The lesion was removed completely with a clear margin of normal surrounding tissue. The patient has made a good recovery from the operation.

Stable Disease to RECIST (7+ months).

Patient 068 (220) Lung Cancer

Male (60 years)

[0267] In May 2011 was diagnosed with T3 N3 M1b non-small cell carcinoma of the right lung (adenocarcinoma), EGFR wild type, in February 2013. Received 10 Cycles of pemetrexed and cisplatin from February to December 2013, followed by thoracic palliative radiotherapy. From January to May 2014 enrolled in the POPLAR study on the docetaxel arm.

[0268] Commenced on NUC-1031 on 3rd October 2014 on 825 mg/m² weekly and received 2 Cycles. End of Cycle 2 CT scan showed Progressive Disease. Removed from study. Progressive Disease.

Patient 069 (221) Colorectal Cancer

Female (45 years)

[0269] In May 2011 was diagnosed with colorectal cancer. In August 2011 received neo-adjuvant capecitabine with radiotherapy and then primary debulking surgery in December 2011. Received adjuvant FOLFOX from January to July 2012. In April 2013 developed a solitary lung recurrence, which was resected. July 2013, a right parieto-occipital recurrence

was found and removed, along with some dermal and subcutaneous cancer deposits. Commenced on cetuximab with FOLFIRI from September 2013 and remained on maintenance cetuximab until September 2014. Gamma-knife treatment in September 2014 for brain metastasis. Is asymptomatic for neurological symptoms.

[0270] Commenced on NUC-1031 on 9th October 2014 on 825 mg/m² weekly and received 3 Cycles. PET scan on 30/10 showed Partial Response. During pre C2 examination cutaneous and sub cutaneous metastases were greatly reduced or almost vanished and no new ones have appeared. End of C2 CT scan showed Stable Disease to RECIST with an 11% reduction in tumour volume from baseline. Developed neutropenia (G4) and leukopenia (G3) during Cycle 3 and dose reduced to 750 mg/m² for Cycle 4. End of C4 CT scan showed Stable Disease to RECIST with 26% reduction in tumour volume from baseline. Following Cycle 4 D1 developed neutropenia and leukopenia, G3 and was dose reduced to 675mg/m² for Cycle 4 D8. Dose was reduced to 625 mg/m² for C5 D8 due to neutropenia and leukopenia, G2. Experiencing visual disturbances, CT scan showed lesion in brain had increased. This has been removed with cyberknife. She will recommence on study drug, C7 D1 on 29th April 2015 at the reduced dose of 500 mg/m².

Stable Disease to RECIST (7+ months)

REFERENCES CITED IN THE DESCRIPTION

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PATENTKRAV

1. NUC-1031 (gemcitabin-[phenyl-benzoxy-L-alaninyl])-phosphat) til anvendelse i målretning af cancerstamceller i behandlingen af cancer.
2. NUC-1031 til anvendelse ifølge krav 1 i behandling af recidiverende eller refraktær
5 cancer hos en human patient.
3. NUC-1031 til anvendelse ifølge krav 2 i behandling af recidiverende cancer hos en human patient.
4. NUC-1031 til anvendelse ifølge et hvilket som helst foregående krav, hvor målretning af cancerstamceller forårsager deres død.
- 10 5. NUC-1031 til anvendelse i behandling af cancer ifølge et hvilket som helst foregående krav ved at reducere eller forebygge cancerudvikling.
6. NUC-1031 til anvendelse i behandling af cancer ifølge et hvilket som helst foregående krav ved at reducere eller forebygge cancerprogression.
7. NUC-1031 til anvendelse i behandling af cancer ifølge et hvilket som helst foregående
15 krav ved at reducere eller forebygge cancerrecidiv.
8. NUC-1031 til anvendelse i behandling af cancer ifølge et hvilket som helst foregående krav ved at reducere eller forebygge cancerspredning.
9. NUC-1031 til anvendelse ifølge et hvilket som helst foregående krav til behandling af
20 en cancer udvalgt fra gruppen bestående af: leukæmi, lymfom, multipelt myelom, lungecancer, levercancer, brystcancer, cancer i hoved og hals, neuroblastom, thyroideakarcinom, hudcancer (herunder melanom), oralt pladeepitelkarcinom, blærecancer, Leydig-celletumor, galdevejscancer, såsom cholangiokarcinom eller galdevejscancer, pankreascancer, coloncancer, kolorektal cancer, osteosarkom og gynækologiske cancere, herunder ovariecancer, endometriecancer, æggeledercancer, uteruscancer og cervixcancer.
- 25 10. NUC-1031 til anvendelse ifølge krav 9, hvor leukæmien er udvalgt fra gruppen bestående af akut lymfoblastisk leukæmi, akut myelogen leukæmi (også kendt som akut myeloid leukæmi eller akut non-lymfocytisk leukæmi), akut promyelocytisk leukæmi, akut lymfocytisk leukæmi, kronisk myelogen leukæmi (også kendt som kronisk myeloid leukæmi, kronisk myelocytisk leukæmi eller kronisk granulocytisk leukæmi), kronisk lymfocytisk leukæmi, monoblastisk leukæmi og hårcelleleukæmi, navnlig akut lymfoblastisk leukæmi.
- 30 11. NUC-1031 til anvendelse ifølge krav 9, hvor lymfomet er udvalgt fra gruppen bestående af: Hodgkins lymfom; non-Hodgkin lymfom; Burkitts lymfom og småcellet lymfocytisk lymfom.

12. NUC-1031 til anvendelse ifølge et hvilket som helst foregående krav ved en ugentlig dosis på mellem 250 mg/m^2 og 1000 mg/m^2 .
13. NUC-1031 til anvendelse ifølge krav 12 ved en ugentlig dosis på mellem 375 mg/m^2 og 900 mg/m^2 .
- 5 14. NUC-1031 til anvendelse ifølge krav 13 ved en ugentlig dosis på mellem 500 mg/m^2 og 825 mg/m^2 .
- 10 15. Fremgangsmåde til bestemmelse af, om en patient med cancer eller en præcancerøs tilstand vil få gavn af forebyggelse eller behandling af cancer med NUC-1031, hvor fremgangsmåden omfatter: test af en biologisk prøve, der er repræsentativ for cancer eller en præcancerøs tilstand hos patienten, for tilstedeværelse af CSC; hvor tilstedeværelsen af CSC i den biologiske prøve indikerer, at patienten vil have gavn af behandling med NUC-1031.