

Combination therapy

Related application

[0001] This application claims priority from Australian provisional application 2023900354, filed on 14 February 2024, the entire disclosure of which is incorporated herein by reference.

Field of the invention

[0002] The present invention is directed to a combination treatment regimen of a FAK inhibitor (FAKi) with a FOLFIRINOX chemotherapy treatment regimen, and the use of that treatment regimen as a means of treating cancer in a patient in need thereof, particularly patients with fibrotic cancers/tumours such as pancreatic cancer.

Background of the invention

[0003] One of the barriers to therapeutic efficacy of chemotherapy in the case of solid tumours is the cellular and stromal complexity of the tumour. The fibrotic response in the tumour microenvironment is termed desmoplasia and is characterised by excessive turnover and remodelling of the extracellular matrix (ECM). In fibrous tumours, such as pancreatic cancer (and specifically pancreatic ductal adenocarcinoma (PDAC)), the increased collagen production and crosslinking not only drives disease progression, and immune suppression but it can adversely affect treatment response and chemotherapeutic efficacy.

[0004] Standard treatment options in these patients are currently limited to gemcitabine combined with nab-paclitaxel, and FOLFIRINOX. But the average 5-year survival rate has remained largely unchanged over the last four decades at ~11%. Therefore, there remains a need for a new combination therapy to improve the efficacy of chemotherapeutics, especially FOLFIRINOX, compared to its efficacy when used on its own.

[0005] Focal Adhesion Kinase (FAK) is often hyperactivated and overexpressed in aggressive cancers, promoting stromal remodelling and inducing tissue stiffness which can accelerate cancer cell proliferation, survival and chemoresistance. Accordingly efforts to improve the efficacy of gemcitabine combined with nab-paclitaxel via stromal manipulation to render tumours more sensitive to the chemotherapy have included use of a FAK inhibitor (Le Large et al. J Exp Clin Cancer Res (2021) 40 (91); and Murphy et al. Biochem. Soc. Trans (2022) 50(4)). But to the extent that a synergistic effect of FAK inhibition was seen, the FAK inhibitor was shown to synergise with the nab-paclitaxel (a microtubule inhibitor) but not gemcitabine (an antimetabolite).

[0006] FOLFIRINOX is a 4-drug chemotherapy regimen first approved for treatment of advanced pancreatic cancer, but also being investigated to treat bowel and other cancers. It is made up of the following four drugs – none of which are microtubule inhibitors but serve to disrupt DNA replication in the same manner as gemcitabine does:

- FOL – folinic acid (also known as calcium folinate or leucovorin);
- F – fluorouracil (5-FU);
- IRIN – irinotecan (Camptosar); and
- OX – oxaliplatin (Eloxatin).

[0007] Accordingly, efforts to date have focused on other drug combinations in an effort to enhance the efficacy of FOLFIRINOX via stromal remodelling.

[0008] For example, IPI-926, an oral Hedgehog inhibitor that can deplete tumour-associated stroma was tested in combination with FOLFIRINOX in patients with advanced pancreatic cancer. Not only did treatment fail to result in consistent increases in tumour perfusion, but a separate phase II trial of IPI-926 plus gemcitabine indicated detrimental effects of that combination (Ko AH, Pancreas. 2016 Mar;45(3)). Hyaluronic acid (HA) is a major component of the extracellular matrix of the tumour stroma. But a trial combining FOLFIRINOX with a pegylated form of human recombinant hyaluronidase (which depletes HA in the cancer extracellular matrix) caused increased toxicity and resulted in decreased treatment duration compared with FOLFIRINOX alone (Ramanathan RK, et al. J Clin Oncol. 2019 May 1;37(13):1062-1069).

[0009] Collectively, the studies to date raised serious questions as to whether (a) stromal modification by any means could enhance the efficacy of FOLFIRINOX; and (b) stromal modification was a worthwhile avenue of investigation when the chemotherapeutic relied on disrupting DNA replication rather than microtubule inhibition.

[0010] Reference to any prior art in the specification is not an acknowledgment or suggestion that this prior art forms part of the common general knowledge in any jurisdiction or that this prior art could reasonably be expected to be understood, regarded as relevant, and/or combined with other pieces of prior art by a skilled person in the art.

Summary of the invention

[0011] Accordingly, in a first embodiment, there is provided a method of treating cancer in a subject in need thereof, the method comprising administering a combination of a FAK inhibitor (FAKi) and a FOLFIRINOX chemotherapy treatment regimen.

[0012] In an alternative embodiment, there is provided a combination of a FAK inhibitor (FAKi) and a FOLFIRINOX chemotherapy treatment regimen for use in the treatment of cancer in a patient in need thereof.

[0013] In a further embodiment, there is provided the use of a FAK inhibitor (FAKi) and a FOLFIRINOX chemotherapy treatment regimen in the manufacture of a medicament for treating cancer in a patient in need thereof.

[0014] In a further embodiment, there is provided the use of a FAK inhibitor (FAKi) in the manufacture of a medicament for treating cancer in a patient in need thereof by combination therapy employing the FAKi with FOLFIRINOX.

[0015] In yet another embodiment there is provided:

- a method of modifying a FOLFIRINOX treatment regimen
- a method of reducing/lessening the symptoms of a FOLFIRINOX treatment regimen
- a method to improve the therapeutic efficacy of FOLFIRINOX compared to its efficacy when used on its own
- a method to improve survival times and quality of life in patients who would normally have received FOLFIRINOX chemotherapy on its own

each method comprising administering a combination of a FAK inhibitor (FAKi) and a FOLFIRINOX chemotherapy treatment regimen. The combination achieves a better outcome than the FOLFIRINOX chemotherapy treatment regimen on its own.

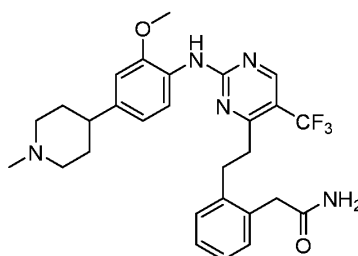
[0016] The combination of a FAK inhibitor (FAKi) and a FOLFIRINOX chemotherapy treatment regimen can be administered in each of the embodiments of the invention in the following ways:

- the FAKi may be administered in a pulsed-dosing regimen that is ceased prior to commencing the FOLFIRINOX chemotherapy treatment.
- the FAKi may be administered in a pulsed-dosing regimen prior to commencing the FOLFIRINOX chemotherapy treatment, and continued once FOLFIRINOX chemotherapy treatment commences.
- the FAKi may be administered concurrently with the FOLFIRINOX chemotherapy treatment and may or may not be continuously administered throughout the treatment cycle.

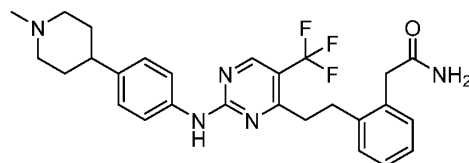
[0017] Administration of the FAKi in a pulsed-dosing regimen that is ceased prior to commencing the FOLFIRINOX chemotherapy treatment is preferred, preferably wherein the FAKi has been administered for a period of at least 3, 4, 5, 6, 7 or 8 days prior to commencement of FOLFIRINOX chemotherapy treatment.

[0018] In each of the above list treatment options, the subject may be chemotherapy naive, or may have had previous chemotherapy treatments, which may include previous FOLFIRINOX chemotherapy treatment.

[0019] In each of the embodiments of the invention, the FAK inhibitor (FAKi) may be defined by formula (I) or formula (II), or a pharmaceutically acceptable derivative thereof:



Formula (I)



Formula (II)

[0020] In preferred embodiments, the FAK inhibitor of Formula (I) and (II) is a tartrate salt.

[0021] In the embodiments of the invention, the cancer is a solid cancer or tumour, and preferably a fibrous cancer or tumour, such as pancreatic cancer, ovarian cancer, colon cancer, rectal cancer, fibrosarcoma and solitary fibrous tumours. In embodiments where the cancer is pancreatic cancer, it is more particularly pancreatic ductal adenocarcinoma (PDAC).

[0022] Further aspects of the present invention and further embodiments of the aspects described in the preceding paragraphs will become apparent from the following description, given by way of example and with reference to the accompanying drawings.

Brief description of the drawings

[0023] Figure 1. Exemplary pulsed-dosing regimen of a FAK inhibitor and FOLFIRINOX for the subcutaneous (Figure 1A) and orthotopic (Figure 1B) injection of tumour cell lines in mice. The mice were treated with FAK inhibitor on days 1 (PM), 2 (AM & PM), 3 (AM & PM) and 4 (AM). The mice were further treated with FOLFIRINOX on days 8&9, with oxaliplatin (AM) and irinotecan (PM) on day 8 and leucovorin calcium (AM) and fluorouracil (PM) on day 9.

[0024] Figure 2. Kaplan-Meier analysis of survival of mice with TKCC10lo patient derived subcutaneous tumours treated with Example FAK inhibitor AMP945/Saline (first trace), Vehicle/Saline (second trace), Vehicle/FOLFIRINOX (third trace) and Example FAK inhibitor AMP945/FOLFIRINOX (fourth trace). Kaplan-Meier curves were compared using log-rank Mantel-Cox test. ns, $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

[0025] Figure 3. Kaplan-Meier analysis of survival of mice with TKCC10lo patient derived orthotopic (intrapancratic) tumours treated with Vehicle/Saline (first trace), Example FAK inhibitor AMP945/Saline (second trace), Vehicle/FOLFIRINOX (third trace) and Example FAK inhibitor AMP945/FOLFIRINOX (fourth trace). Kaplan-Meier curves were compared using log-rank Mantel-Cox test. ns, $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[0026] Figure 4. Tumour Volume (mm^3) at endpoint (Figure 4A) and over time in days (B) for mice treated with FOLFIRINOX monotherapy or primed with AMP945 prior to FOLFIRINOX. Tumour volume of mice treated with FOLFIRINOX monotherapy shows higher tumour volume.

[0027] Figure 5. A) Cleaved Caspase-3 positive cells (%) for FOLFIRINOX and the combination therapy of a FAK inhibitor and FOLFIRINOX. B) Ki67 positive cells (%) for FOLFIRINOX and the combination therapy of a FAK inhibitor and FOLFIRINOX. N= 5 mice/tumours per treatment group and 6 Regions of Interest for each IHC stain per tumour. Results are mean $\pm$ SEM. P-values determined using an un-paired *t* test with Welch correction for unequal variance. Unless otherwise stated, all significance is compared to FOLFIRINOX ns, $P > 0.05$, * $P < 0.05$

Definitions

[0028] For purposes of interpreting this specification, terms used in the singular will also include the plural and vice versa.

[0029] As used herein, except where the context requires otherwise, the term "comprise" and variations of the term, such as "comprising", "comprises" and "comprised", are not intended to exclude further additives, components, integers or steps.

[0030] "About" as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of $\pm 5\%$, in some instances $\pm 1\%$, and in some instances $\pm 0.1\%$ from the specified value, as such variations are understood by the skilled person to be appropriate to perform the disclosed methods.

[0031] Throughout this disclosure, various aspects of the disclosure can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosure. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

[0032] The term "FOLFIRINOX" as used herein refers generally to the combination chemotherapy comprising folinic acid (also known as calcium folinate or leucovorin), 5 fluorouracil, irinotecan hydrochloride and oxaliplatin. Modified versions of FOLFIRINOX are contemplated to be encompassed by the use of this term throughout the specification. For example NALIRIFOX is FOLFIRINOX wherein the irinotecan is a liposomal formulation. Other examples include FOLFOX or FOLFIRI.3, as well as combinations of FOLFIRINOX variations such as FIRGEM, NAB-FOLFIRI, NALIRIFOX and NAB-FOLFOX. Provided the chemotherapy has those 4 components, it is considered to be FOLFIRINOX for the purposes of this invention.

[0033] FOLFIRINOX is described herein in terms of being a chemotherapy, a chemotherapy treatment, chemotherapy regimen, a chemotherapy treatment regimen and a treatment regimen. These terms are interchangeable as the skilled person is well aware that FOLFIRINOX is not a single drug or formulation.

[0034] By "combined therapy" or "combination therapy" as used herein it is meant that the treatment steps involve administration of both a FAKi and FOLFIRINOX. The administration can

be concurrent – either administered at the same time, or as a single formulation/medicament. Alternatively, the FAKi and FOLFIRINOX can be administered sequentially. By sequential it is intended to mean any time frame between the administration of the first and second component, provided that the effects of the first component are still sufficient to positively impact the effects of the second component.

[0035] As would be further understood by the skilled person from the context of the specification as a whole, the components of the “combined therapy” or “combination therapy” are preferably formulated as two separate formulations or medicaments.

[0036] By “pulsed dosing” regimen it is meant administration of the FAKi for a period of time that is commenced prior to the administration of the FOLFIRINOX. The FAKi may be ceased prior to any round of administration of the FOLFIRINOX, or continued once FOLFIRINOX administration begins. The pulsed dosing regimen may also involve a combination. For example dosing with FAKi and ceasing prior to administration of the first round of FOLFIRINOX; then dosing with FAKi and continuing upon commencement of the second round of FOLFIRINOX.

[0037] The term “pharmaceutically acceptable” as used herein pertains to compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgement, suitable for use in contact with the tissues of a subject (e.g. human) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, excipient, etc. must also be “acceptable” in the sense of being compatible with the other ingredients of the formulation.

[0038] As used herein, the term “pharmaceutically acceptable salt” refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge et al., describe pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 1977, 66, 1–19, incorporated herein by reference. Pharmaceutically acceptable salts of the compounds of this invention include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate,

glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like.

[0039] As used herein, "pharmaceutically acceptable excipient" means a pharmaceutically acceptable material which is included in the composition for a purpose other than pharmaceutical efficacy (this is not intended to exclude materials which may have some biological effect).

[0040] As used herein, "preventing" or "prevention" is intended to refer to at least the reduction of likelihood of the risk of (or susceptibility to) acquiring a disease or condition (i.e., causing at least one of the clinical symptoms of the disease not to develop in an individual that may be exposed to or predisposed to the disease but does not yet experience or display symptoms of the disease). Biological and physiological parameters for identifying such patients are provided herein and are also well known by physicians. The skilled artisan will appreciate that "prevention" is not an absolute term. In particularly preferred embodiments, the methods of the present invention can be to prevent or reduce the severity, or inhibit or minimize progression, of a symptom of a disease or condition as described herein. As such, the methods of the present invention have utility as treatments as well as prophylaxes.

[0041] The terms "treatment" or "treating" of a subject includes delaying, slowing, stabilising, curing, healing, alleviating, relieving, altering, remedying, less worsening, ameliorating, improving, or affecting the disease or condition, the symptom of the disease or condition, or the risk of (or susceptibility to) the disease or condition. The term "treating" refers to any indication of success in the treatment or amelioration of an injury, pathology or condition, including any objective or subjective parameter such as abatement; remission; lessening of the rate of worsening; lessening severity of the disease; stabilization, diminishing of symptoms or making the injury, pathology or condition more tolerable to the individual; slowing in the rate of degeneration or decline; making the final point of degeneration less debilitating. Treatment may not necessarily result in the complete clearance of a disease or disorder but may reduce or minimise complications and side effects of infection and the progression of a disease or disorder. The success or otherwise of treatment may be monitored by, amongst other things, physical examination of the individual, CT scan, MRI, or blood biomarkers.

[0042] The term "therapeutically-effective amount," as used herein, pertains to that amount of an active compound, or a material, composition or dosage form comprising an active

compound, or a treatment regime and the components thereof, which is effective for producing some desired therapeutic effect, commensurate with a reasonable benefit/risk ratio, when administered in accordance with a desired treatment regimen.

Detailed description of the embodiments

[0043] Reciprocal interactions between the tumour and stroma are mediated by bidirectional integrin-mediated signalling, in particular by Focal Adhesion Kinase (FAK). FAK is often hyperactivated and overexpressed in aggressive cancers, promoting stromal remodelling and inducing tissue stiffness which can accelerate cancer cell proliferation, survival and chemoresistance. FAK inhibitors (FAKi), such as the class of 2,4,5-substituted pyrimidines described in WO2012110774, have application on their own for the reduction of cell adhesion, cell migration, cell invasion, cell proliferation and chemo-resistance. Furthermore, a FAK inhibitor has applicability to induce apoptosis for cells in inappropriate extra cellular matrix environments and reduce angiogenesis.

[0044] The present inventors have surprisingly found that stromal manipulation (or 'priming') via short-term FAK inhibition using particular FAK inhibitor compounds, can render pancreatic ductal adenocarcinoma (PDAC) cells and other solid tumours more vulnerable and therefore more responsive to subsequent FOLFIRINOX chemotherapy with unexpectedly improved survival times in animal models compared to other FAKi/FOLFIRINOX combinations. This is despite previous studies in the field that showed (a) synergism between other FAK inhibitors and chemotherapeutics was via a mechanism not relevant to FOLFIRINOX and (b) stromal modification in many instances was detrimental to the efficacy of FOLFIRINOX and other chemotherapeutics (gemcitabine) that rely on disrupting DNA replication.

[0045] FOLFIRINOX is a chemotherapy regimen, typically used for the treatment of advanced pancreatic cancer. It is made up of FOL – folinic acid (leucovorin), F – fluorouracil, IRIN – irinotecan and OX – oxaliplatin.

- leucovorin is converted to another reduced folate, 5,10-methylenetetrahydrofolate, which stabilises the binding of FdUMP (the pharmacologically active form of fluorouracil) to thymidylate synthase and thereby enhances the inhibition of thymidylate synthase by FdUMP.
- Fluorouracil is metabolised to 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP), which is an inhibitor of thymidylate synthase, an enzyme important in DNA repair and replication.

- Irinotecan and its active metabolite SN-38 bind to the topoisomerase I-DNA complex and prevent religation of DNA single-strand breaks, in turn leading to double-strand DNA breaks and cytotoxicity.
- Oxaliplatin, by displacement of its oxalate ligand, is able to form inter- and intra-strand crosslinks in DNA. These crosslinks inhibit DNA replication and transcription causing cytotoxicity.

[0046] In a first embodiment of the invention there is provided a method of treating cancer in a subject in need thereof, the method comprising administering a combination of a FAK inhibitor (FAKi) and a FOLFIRINOX treatment regimen.

[0047] The invention further provides a combination of a FAK inhibitor and a FOLFIRINOX chemotherapy treatment regimen for use in the treatment of cancer in a patient in need thereof.

[0048] The invention further provides the use of a FAK inhibitor in the manufacture of a medicament to be administered to a subject with cancer, wherein following the administration of the FAK inhibitor, the subject is to be administered a FOLFIRINOX treatment regimen.

[0049] In a further embodiment, there is provided the use of a FAK inhibitor (FAKi) in the manufacture of a medicament for treating cancer in a patient in need thereof by combination therapy employing the FAKi with FOLFIRINOX.

[0050] The combination of a FAK inhibitor (FAKi) and a FOLFIRINOX chemotherapy treatment regimen can be administered in each of the embodiments of the invention in the following ways:

- the FAKi may be administered in a pulsed-dosing regimen that is ceased prior to commencing the FOLFIRINOX chemotherapy treatment.
- the FAKi may be administered in a pulsed-dosing regimen prior to commencing the FOLFIRINOX chemotherapy treatment, and continued once FOLFIRINOX chemotherapy treatment commences.
- the FAKi may be administered concurrently with the FOLFIRINOX chemotherapy treatment. "Concurrent" in this embodiment can be either at the same time (eg oral administration of the FAKi and intravenous infusion of the FOLFIRINOX chemotherapy) or concurrent if the FAKi is formulated with a component of the FOLFIRINOX chemotherapy.

[0051] The above administration options can also be combined over multiple rounds of a treatment regimen. For example, for the first round of treatment, the FAKi may be administered in a pulsed-dosing regimen that is ceased prior to commencing the FOLFIRINOX chemotherapy treatment, and for the second round of treatment, the FAKi may be administered in a pulsed-dosing regimen prior to commencing the FOLFIRINOX chemotherapy treatment, and continued once FOLFIRINOX chemotherapy treatment commences.

[0052] Administration of the FAKi in a pulsed-dosing regimen that is ceased prior to commencing the FOLFIRINOX chemotherapy treatment is preferred, preferably wherein the FAKi has been administered for a period of at least 3, 4, 5, 6, 7 or 8 days prior to commencement of FOLFIRINOX chemotherapy treatment. Accordingly, in a preferred embodiment of the invention there is provided a method of treating cancer in a subject in need thereof, the method comprising administering a combination of a FAK inhibitor (FAKi) and a FOLFIRINOX treatment regimen, wherein the FAKi is administered to the subject for at least 3 days, preferably for 4-7 days, and is ceased prior to the commencement of the FOLFIRINOX treatment regimen.

[0053] In these embodiments, as detailed below, the FOLFIRINOX treatment regimen is the U.S. Food and Drug Administration approved "standard" FOLFIRINOX treatment regimen for use in treating patients with pancreatic cancer.

[0054] The combined therapy FAKi/FOLFIRINOX is expected to be synergistic and improve the therapeutic efficacy of FOLFIRINOX compared to its efficacy when used on its own. The improvement in therapeutic efficacy and treatment outcomes may be assessed as, or evidenced by, one of more of:

- improved survival times probabilities and quality of life in patients who would normally have received FOLFIRINOX treatment regimen on its own; and/or
- modifications of the standard FOLFIRINOX treatment regimen; and/or
- fewer or less severe side effects of the standard FOLFIRINOX treatment regimen including reducing the degree of toxicity.

[0055] When reference is made to the standard FOLFIRINOX treatment regimen it is a reference to the following regimen:

Oxaliplatin	85 mg/m ² intravenous (I.V.) infusion is given over 2 hours on Day 1
Folinic acid	400 mg/m ² I.V. infusion is given over 2 hours on Day 1 simultaneously with oxaliplatin
Irinotecan	180 mg/m ² I.V. infusion is given over 90 minutes on Day 1
Fluorouracil	400 mg/m ² I.V. push over 3 to 5 minutes on Day 1 (optional); 2400 mg/m ² continuous I.V. infusion over 46 hours beginning Day 1

[0056] The treatment regimen is repeated as a 2 week cycle, typically for up to 6 months ie 12 cycles.

[0057] Accordingly, in one embodiment of the invention, there is provided a method of modifying the standard FOLFIRINOX treatment regimen in a subject in need of FOLFIRINOX treatment comprising the step of

- (a) administering a FAK inhibitor (FAKi) to the subject for at least 3 days;
- (b) optionally ceasing administration of the FAKi; and
- (c) commencing a modified FOLFIRINOX treatment regimen,

wherein the modification is a reduction in the dose for one or more of the 4 components of FOLFIRINOX and/or a reduction in the amount of time one or more of the 4 components of FOLFIRINOX is infused and/or a reduction in the treatment frequency or total treatment cycles.

[0058] Any such modifications are envisaged to be advantageous to the patient in terms of treatment time and treatment side effects (including lower toxicity), improved patient compliance and to the cost of the treatment when less is used.

[0059] In an alternative embodiment there is provided a method of lessening the incidence or severity of side effects experienced by subjects compared to those experienced when receiving FOLFIRINOX alone, the method comprising administering a combination of a FAK inhibitor (FAKi) and a FOLFIRINOX treatment regimen, wherein the FAKi is administered to the subject for at least 3 days, and is ceased prior to the commencement of the FOLFIRINOX treatment regimen. The FOLFIRINOX treatment regimen may be the standard FOLFIRINOX treatment or the modified FOLFIRINOX wherein the modification is a reduction in the dose for one or more of the 4 components of FOLFIRINOX and/or a reduction in the amount of time one or more of the 4 components of FOLFIRINOX is infused and/or a reduction in the treatment frequency or total treatment cycles.

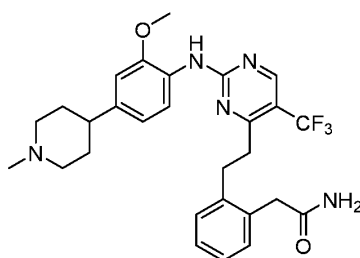
[0060] The side effects that are lessened or reduced include, without limitation, neutropenia, fatigue, headache, nausea, vomiting, diarrhoea, loss of appetite, low platelets, numbness and

tingling, anaemia, decreased liver function, acute cholinergic syndrome, blood clots and neutropenic fever. Without being bound by any theory, it is envisaged that the reduction or lessening of these symptoms will be as a result of the FOLFIRINOX treatment regimen being modified to have lower doses and/or decreased infusion times and/or less frequent or fewer treatment cycles. The degree of lessening and reduction may also be deemed prevention of the progression of the symptoms and side effects.

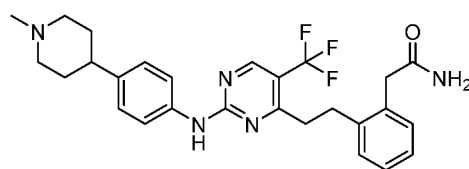
[0061] In embodiments wherein the FAKi is administered as a pulsed-dosing regimen for a period of time, then ceased before the FOLFIRINOX treatment regimen is commenced, preferably, the FAKi is administered for at least 3, 4, 5, 6, 7 or 8 days, more preferably 4-7 days.

The FAKi used in the embodiments of the invention

[0062] The FAK inhibitor may be defined by Formula (I) or (II) or a pharmaceutically acceptable derivative thereof:



Formula (I) (also referred to as AMP945 and narmafotinib in the examples)



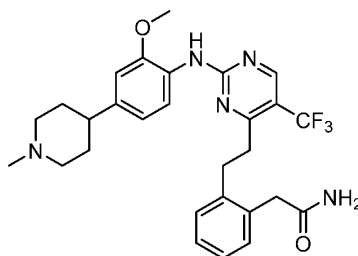
Formula (II)

[0063] In embodiments, the FAK inhibitor of Formula (I) and (II) is a tartrate salt.

[0064] In embodiments, the tartrate salt may be D-tartrate or L-tartrate, preferably L-tartrate.

[0065] Accordingly, in a preferred embodiment of the invention there is provided a method of treating cancer in a subject in need thereof, the method comprising administering a combination of a FAK inhibitor (FAKi) and a FOLFIRINOX treatment regimen, wherein the FAKi is administered to the subject for at least 3 days, preferably 4-7 days, and is ceased prior to the

commencement of the FOLFIRINOX treatment regimen, wherein the FAKi is defined by formula (I) or a pharmaceutically acceptable derivative or salt thereof:



Formula (I).

[0066] Preferably, the FAKi is a tartrate salt.

The conditions to be treated by the FAKi/FOLFIRINOX combination therapy

[0067] The combination therapy of the present invention can be used in the treatment of proliferative diseases, in particular as an anticancer therapy.

[0068] In embodiments, the cancer is selected from a solid cancer, including but not limited to bone cancer, brain stem glioma, breast cancer, cancer of the adrenal gland, cancer of the anal region, cancer of the bladder, cancer of the endocrine system, cancer of the oesophagus, cancer of the head or neck, cancer of the kidney or ureter, cancer of the liver, cancer of the parathyroid gland, cancer of the penis, cancer of the small intestine, cancer of the thyroid gland, cancer of the urethra, carcinoma of the cervix, carcinoma of the endometrium, carcinoma of the fallopian tubes, carcinoma of the renal pelvis, carcinoma of the vagina, carcinoma of the vulva, colon cancer, cutaneous or intraocular melanoma, fibrosarcoma, lung cancer, lymphocytic lymphomas, neoplasms of the central nervous system (CNS), ovarian cancer, pancreatic cancer, pituitary adenoma, primary CNS lymphoma, prostate cancer, rectal cancer, renal cell carcinoma, sarcoma of soft tissue, skin cancer, spinal axis tumours, solitary fibrous tumour, stomach cancer and uterine cancer.

[0069] In preferred embodiments the cancers are fibrous cancer and tumours, such as pancreatic cancer, ovarian cancer, colon cancer, rectal cancer, fibrosarcoma and solitary fibrous tumours. In embodiments where the cancer is pancreatic cancer, it is more particularly pancreatic ductal adenocarcinoma (PDAC).

[0070] In embodiments, the FAK inhibitor is administered in a dose between 10mg and 1 g; preferably in a dose between 50mg and 600mg, and most preferably in a dose between 100mg and 400mg.

[0071] Any type of cell may be treated, including but not limited to, lung, gastrointestinal (including, e.g., bowel, colon, rectal), breast (mammary), ovarian, prostate, liver (hepatic), kidney (renal), bladder, pancreas, brain, and skin.

Administration

[0072] The FAKi compound or pharmaceutical composition comprising the active compound may be administered to a subject by any convenient route of administration, whether systemically/ peripherally or at the site of desired action, including but not limited to, oral (e.g. by ingestion); and parenteral administration (e.g. by injection, including cutaneous, subcutaneous, intramuscular, intravenous and intradermal).

[0073] Standard and modified FOLFIRINOX treatment is by parenteral administration.

[0074] The subject may be a eukaryote, an animal, a vertebrate animal, a mammal, a rodent (e.g. a guinea pig, a hamster, a rat, a mouse), murine (e.g. a mouse), canine (e.g. a dog), feline (e.g. a cat), equine (e.g. a horse), a primate, simian (e.g. a monkey or ape), a monkey (e.g. marmoset, baboon), an ape (e.g. gorilla, chimpanzee, orang-utan, gibbon), or a human.

Formulations

[0075] While it is possible for the FAKi in the combination therapy to be administered alone, it is preferable to present it as a pharmaceutical composition (e.g. formulation) comprising at least one FAKi, as defined above, together with one or more pharmaceutically acceptable carriers, adjuvants, excipients, diluents, fillers, buffers, stabilisers, preservatives, lubricants, or other materials well known to those skilled in the art and optionally other therapeutic or prophylactic agents.

[0076] Thus, the present invention further provides pharmaceutical compositions for use in the methods of the invention, as defined above, and methods of making a pharmaceutical composition comprising admixing at least one FAKi, as defined above, together with one or more pharmaceutically acceptable carriers, excipients, buffers, adjuvants, stabilisers, or other materials, as described herein.

[0077] The FAKi may also be combined in a formulation with FOLFIRINOX for parenteral administration.

[0078] Suitable carriers, excipients, etc. can be found in standard pharmaceutical texts, for example, Remington's Pharmaceutical Sciences, 18th edition, Mack Publishing Company, Easton, Pa., 1990.

[0079] 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, sulphanilic, aspartic, glutamic, edetic, stearic, palmitic, oleic, lauric, pantothenic, tannic, ascorbic and valeric acids.

[0080] General information on types of pharmaceutically acceptable salts and their formation is known to those skilled in the art and is as described in general texts such as "Handbook of Pharmaceutical salts" P. H. Stahl, C. G. Wermuth, 1st edition, 2002, Wiley-VCH and S. M. Berge et al., J. Pharmaceutical Sciences, 1977, 66, 1–19.

[0081] Basic nitrogen-containing groups may be quarternised with such agents as lower alkyl halide, such as methyl, ethyl, propyl, and butyl chlorides, bromides and iodides; dialkyl sulfates like dimethyl and diethyl sulfate; and others.

[0082] The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the FAKi with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the FAKi with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

[0083] Formulations may be in the form of liquids, solutions, suspensions, emulsions, elixirs, syrups, tablets, lozenges, granules, powders, capsules, cachets, pills, ampoules, suppositories, pessaries, ointments, gels, pastes, creams, sprays, mists, foams, lotions, oils, boluses, electuaries, or aerosols.

[0084] Formulations suitable for oral administration (e.g. by ingestion) may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the tartrate salt; as a powder or granules; as a solution or suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion; as a bolus; as an electuary; or as a paste.

[0085] Preferably, the formulation of the FAKi is suitable for oral administration that can be administered sequentially to the FOLFIRINOX treatment regimen or at the same time as the FOLFIRINOX treatment regimen but as it's own formulation. But it is envisaged that the FAKi could be formulated with one of the components of the FOLFIRINOX treatment regimen for concurrent administration.

[0086] A tablet may be made by conventional means, e.g., compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the FAK inhibitor in a free-flowing form such as a powder or granules, optionally mixed with one or more binders (e.g. povidone, gelatin, acacia, sorbitol, tragacanth, hydroxypropylmethyl cellulose); fillers or diluents (e.g. lactose, microcrystalline cellulose, calcium hydrogen phosphate); lubricants (e.g. magnesium stearate, talc, silica); disintegrants (e.g. sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxy methyl cellulose); surface-active or dispersing or wetting agents (e.g. sodium lauryl sulfate); and preservatives (e.g. methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, sorbic acid). Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the FAK inhibitor therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

[0087] Formulations suitable for parenteral administration (e.g. by injection, including cutaneous, subcutaneous, intramuscular, intravenous and intradermal), include aqueous and non-aqueous isotonic, pyrogen-free, sterile injection solutions which may contain anti-oxidants, buffers, preservatives, stabilisers, bacteriostats, and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. Examples of suitable isotonic vehicles for use in such formulations include Sodium Chloride Injection, Ringer's Solution, or Lactated Ringer's Injection. Typically, the concentration of the tartrate salt in the solution is from about 1 ng/ml to about 10 µg/ml, for example from about 10 ng/ml to about 1 pg/ml. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, and tablets. Formulations may be in the form of liposomes or other microparticulate systems which are designed to target the FAK inhibitor to blood components or one or more organs.

Modification of the FOLFIRINOX regimen

[0088] The standard FOLFIRINOX treatment regimen consists of:

Oxaliplatin	85 mg/m ² intravenous (I.V.) infusion is given over 2 hours on Day 1
Folinic acid	400 mg/m ² I.V. infusion is given over 2 hours on Day 1 simultaneously with oxaliplatin
Irinotecan	180 mg/m ² I.V. infusion is given over 90 minutes on Day 1
Fluorouracil	400 mg/m ² I.V. push over 3 to 5 minutes on Day 1 (optional); 2400 mg/m ² continuous I.V. infusion over 46 hours beginning Day 1

[0089] The treatment regimen is repeated as a 2 week cycle, typically for up to 6 months ie 12 cycles.

[0090] When a subject is treated with a FAKi prior to administration of the standard FOLFIRINOX treatment regimen, it is possible to modify the standard treatment to:

- use less of one or more of the 4 components of FOLFIRINOX and/or
- reduce the infusion time of one or more of the 4 components of FOLFIRINOX and/or
- reduce the treatment frequency and/or
- reduce the total treatment cycles.

Any such modifications are envisaged to be advantageous to the patient in terms of treatment time, patient compliance, and treatment side effects (including lower toxicity), and to the cost of the treatment when less is used.

[0091] Modification can include one or more of the following:

- Reducing oxaliplatin to a dose of 50-80mg/m² and/or
- reducing leucovorin to a dose of 200-350 mg/m² and/or
- reducing irinotecan to a dose of 130-165 mg/m² and/or
- reducing fluorouracil to a dose of 1800-2200 mg/m², 2400 mg/m²

[0092] Preferably, modification involves reducing the number of cycles from 12 to less than 10. For a patient, this reduction in the number of cycles is a month shorter of treatment.

[0093] FOLFIRINOX-related treatment variations, such as FOLFOX or FOLFIRI.3, as well as combinations of FOLFIRINOX variations such as FIRGEM, NAB-FOLFIRI, NALIRIFOX and NAB-FOLFOX are contemplated.

Dosing and dosage of the FAKi

[0094] Administration of the FAK inhibitor is preferably effected in a pulsed-dosing regimen, wherein the FAK inhibitor is provided as a large dose, prior to the administration of the FOLFIRINOX treatment regimen, in one dose or intermittently (e.g. in divided doses at appropriate intervals) throughout the course of treatment.

[0095] Preferably the pulsed dosing regimen involves administration of the FAKi for at least 3, 4, 5, 6, 7 or 8 days, most preferably for 4-7 days, prior to the administration of the FOLFIRINOX treatment regimen.

[0096] In general, a suitable dose of the FAKi is in the range of about 100 pg to about 250 mg per kilogram body weight of the subject per day.

[0097] It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

Examples

[0098] The invention will now be described by reference to the following non-limiting examples.

Example 1 - Preparation of FAK inhibitors

[0099] The FAK inhibitors of the present invention were prepared according to the procedure disclosed in US patent number 9174946 B2, the contents of which are incorporated in its entirety.

Example 2 – Materials and methods**Statistical analysis**

[0100] Statistical analysis was performed using GraphPad Prism (GraphPad Software, Inc., CA) with significance given as; ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$. Following normality conformation using a Shapiro-wilk test, data were analysed using an unpaired t test with Welch's correction for normally distributed data, or a Mann-Whitney test for non-normally distributed data. For data normalized to 1, a one-sample t-test was performed. FUCCI cell cycle data analysis was assessed using a two-way analysis of variance (ANOVA) with Tukey correction for multiple comparisons. Kaplan-Meier curves were compared using a log-rank Mantel-Cox test. For all other datasets, p-values were determined by ordinary one-way

ANOVA with a Tukey correction for multiple comparisons for normally distributed data or a Kruskal-Wallis test with Dunn's multiple comparison for non-normally distributed data.

Animals

[0101] Animal experiments were conducted in accordance with the Garvan/St. Vincent's Animal Ethics Committee guidelines (19/10, 19/13, 22/09, 22/10) and in compliance with the Australian code of practice for care and use of animals for scientific purposes. Mice were kept in IVC isolated cages on a 12 hour light/dark cycle and fed *ab libitum*.

Drug treatment schedules

[0102] Stock solutions of the FAK inhibitor (AMP945/narmafotinib) were prepared at 10 mM in DMSO for *in vitro* utilization at a range of concentrations from 5 to 100 nM in cell culture medium with DMSO as vehicle control. For *in vivo* studies the FAKi was dissolved in, 0.5%w/v Hydroxypropyl methylcellulose, 0.5% benzyl alcohol, and 0.4% Tween 80 in sterile water, and was administered twice daily by oral gavage for 3 days, using a 22-gauge feeding tube (Instech Laboratories, FTP-22-25) coated in sucrose solution (24%, Sigma-Aldrich, S9378).

[0103] In subcutaneous models, treatment with the FAKi (10 mg/kg) commenced 6 days after KPC cell injection (average tumor volume of 30 mm³) and 24-28 days for TKCC10lo cells (average tumor volume of 50 mm³). In pancreatic orthotopic models, FAKi treatment began when tumors were palpable, and detectable by IVIS imaging (average flux of 1x10⁹) 4-weeks post-intrapancreatic injection for TKCC10 cells.

[0104] For FOLFIRINOX, in solution Oxaliplatin (Clifford Hallam Healthcare Pty Ltd, 5 mg/kg), Irinotecan (Clifford Hallam Healthcare Pty Ltd, 25 mg/kg), Calcium Leucovorin (Clifford Hallam Healthcare Pty Ltd 100 mg/kg) and 5-Fluorouracil (Baxter Healthcare Pty Ltd, 25 mg/kg) or saline vehicle were administered by intraperitoneal injection. All treatments were administered as per the treatment schedule outlined in the corresponding examples, figures and figure legends.

Cell culture

[0105] Primary KPC cells have previously been isolated from end-stage Pdx1-Cre, LSL-Kras^{G12D/+}, LSL-Trp53^{R172H/+} KPC mice (J. P. Morton et al., Proc Natl Acad Sci U S A 107, 246-251 (2010); J. P. Morton et al., Gastroenterology 139, 292-303 (2010); and S. R. Hingorani et al., Cancer Cell 7, 469-483 (2005), whilst Telomerase-immortalized fibroblasts (TIFs) were also generated previously (C. Vennin et al., Sci Transl Med 9, (2017); K. J. Murphy et al., Sci Adv 7, eabh0363 (2021); Z. Erami et al., Cell Rep 14, 152-167 (2016); and J. Munro, K. et al.

Oncogene 20, 3541-3552 (2001)). KPC cells and TIFs were maintained in Dulbecco's Modified Eagle Media (DMEM, high glucose, pyruvate, Gibco) supplemented with 10% Fetal Bovine Serum (FBS, Hyclone) and 10 mM HEPES (Gibco). TKCC10lo cells were maintained in 1:1 M199 media / Ham's F12 medium (Gibco) supplemented with 7.5% FBS, 15 mM HEPES, 2 mM Glutamine, 1x MEM vitamins, 25 ng/ml apo-transferrin, 0.2IU/ml insulin, 6.5 mM Glucose, 40 ng/ml Hydrocortisone, 20 ng/ml EGF, Triiodothyronine 0.5 pg/ml and 2 µg/ml O-phosphoryl ethanolamine. All cells were cultured in the presence of Penicillin/Streptomycin (100 U/ml and 100 mg/ml, respectively) and maintained at 37 °C and 5% CO₂. Experiments were conducted at 20% oxygen in a HeraCell 150i CO₂/O₂ incubator for KPC cells and TIFs and at 5% oxygen for TKCC10lo lines. All cell lines were confirmed to be mycoplasma free.

Generation of stable cell lines

[0106] Generation of stable cell lines expressing the pPBDEST-Lyn-FAK biosensor was achieved by co-transfection of the pPBDEST-Lyn-FAK vector and pCMV-hyPBase transposase (obtained from the Wellcome Trust Sanger Institute, (K. J. Murphy et al., Sci Adv 7, eabh0363 (2021); K. Yusa et al. Proc Natl Acad Sci U S A 108, 1531-1536 (2011); and J. Seong et al., Nat Commun 2, 406 (2011)) using Lipofectamine 3000 Reagent as per the manufacturer's instructions. KPC-FUCCI cells were also engineered to express the FUCCI cell cycle reporter (mKO2-hCdt1 and mAG-hGeminin (A. Chou et al., Gut 67, 2142-2155 (2018)) or Luciferase-GFP (pLV430G) using a 3rd generation lentiviral packaging system, as previously achieved. Following transfection and transduction, respectively, positive cells were selected by Fluorescence Activated Cell Sorting (FACS).

Cell derived matrix (CDM) assays

[0107] CDMs were established as previously described (C. Vennin et al., Sci Transl Med 9, (2017); K. J. Murphy et al., Sci Adv 7, eabh0363 (2021); Z. Erami et al., Cell Rep 14, 152-167 (2016); K. J. Murphy et al., Bio Protoc 12, (2022); and E. Cukierman, et al. Science 294, 1708-1712 (2001)). To generate CDMs on glass bottom dishes, the surface was coated with 2% gelatin and allowed to set at 37 °C for 2 hours then rinsed twice with Dulbecco's phosphate buffered saline (PBS) prior to formalin fixation at room temperature for 30 minutes. After two rinses with PBS, fixed gelatin crosslinks were quenched in 1M sterile glycine for 30 minutes at room temperature and coated plates rinsed twice with PBS and once with DMEM prior to TIF cell seeding. TIFs were allowed to expand until confluent. Medium supplemented with ascorbic acid (50 mg/mL) was then refreshed every other day for 7 days, when TIFs were removed (Extraction Buffer: 0.5% v/v Triton X-100, 20 mM Ammonium Hydroxide, 1% w/v Sodium Deoxycholate). CDMs were rinsed with PBS and DMEM prior to seeding of cancer cells.

Organotypic Invasion assay

[0108] *Collagen Extraction:* Collagen I was extracted from rat tails as previously described (C. Vennin et al., Sci Transl Med 9, (2017); K. J. Murphy et al., Sci Adv 7, eabh0363 (2021); and C. Vennin et al., Nat Commun 10, 3637 (2019)). Briefly, once rat tails were defrosted, pronged tweezers were used to pull collagen tendons free of the epithelium and skeletal structure. Extracted tendons from 10-12 tails were then solubilized in 1500 mL of 0.5 M acetic acid for 48-72 hours at 4 °C on a magnetic stirrer. The mixture was then filtered through a strainer to remove sheath, prior to precipitation with 10% (w/v) sodium chloride on a magnetic stirrer over 6-8 hours. Once an opaque homogenous white solution was formed, the mixture was centrifuged for 30 minutes at 4 °C at 10,000 rpm. The resulting collagen precipitate was dissolved in 400-600 mL of 0.25 M acetic acid at 4 °C on a magnetic stirrer overnight and the solution dialyzed in 4 L of 17.4 mM acetic acid for 4 days with acid refreshed every 12 hours.

[0109] *Contraction Assays:* Organotypic matrices were generated as described in (C. Vennin et al., Sci Transl Med 9, (2017); K. J. Murphy et al., Sci Adv 7, eabh0363 (2021); and C. Vennin et al., Nat Commun 10, 3637 (2019)). Briefly, 8×10^4 TIFs/matrix were embedded into acid-extracted rat tail collagen (~2.5 mg/mL) in the presence of 1x MEM, 8.8% FBS and neutralized with sodium hydroxide. Matrices were allowed to set at 37 °C before detachment and allowed to contract for 12 days in DMEM with 10% FBS, 10 mM HEPES and Penicillin/Streptomycin (100 U/ml and 100 mg/ml, respectively). For early treatment (priming), matrices were treated during contraction with vehicle, 5 nM, 10nM, 20 nM, 50 nM or 100 nM FAK inhibitor at day 0 and refreshed at day 6.

[0110] *Invasion Assays:* After 12 days of contraction, vehicle or 20 nM FAK inhibitor was washed out and 1×10^5 KPC cells were seeded onto contracted matrices. After 72 hours of cancer cell growth, seeded matrices were moved to an air-liquid interface on a metal grid and KPC cancer cells allowed to invade into the matrix for 14 days. For late treatment regimes, media was supplemented with vehicle or 20 nM FAK inhibitor and refreshed 3 times per week during cell invasion. Chronic treatment involved FAK inhibitor treatment during both matrix contraction and during invasion. Matrices were then fixed in 10% neutral buffered formalin and processed for paraffin block embedding, sectioning and histological and immunohistochemistry (IHC) analysis (Garvan Histopathology Core Facility). Using cell numbers calculated from H&E staining, cell invasion index was calculated as total number of cells invading divided by the cells on top of the matrix and normalized to average invasion index of vehicle cells.

$$\text{Invasive Index} = \frac{\text{Number of invaded cells}}{\text{Cells on top of and within the matrix}}$$

ICGC and APCI patient data

[0111] For IHC analysis of patient TMAs from the APCI cohort tumor cores of deceased patients (3 per patient) with complete survival datasets (total 158) were scored for stromal integrity (Picrosirius Red) and analyzed using TWOMBLI. For pTyr-397-FAK, DAB intensity was determined using QuPath (2). Kaplan-Meier curves were generated, using GraphPad Prism and a log-rank test performed to determine significance.

Subcutaneous Injections and Intravital Imaging

[0112] 1×10^6 KPC-FAK cells or KPC-FUCCI cells in PBS per mouse were injected into the rear flank of BALB/c-Fox1nuAusb mice whilst under anesthesia (isoflurane 3%, O₂ 1 L/min, with continuous vacuum to remove excess isoflurane). Tumors were allowed to develop for 6 days to an average volume of 30 mm³ for KPC tumors before treatment schedules commenced. For KPC-FAK derived tumors, mice were treated with narmafotinib (10 mg/kg) or vehicle twice daily for three days with the final treatment 4 hours prior to skin flap surgery and subsequent intravital imaging (Figure 4A). For KPC-FUCCI derived tumors, mice were treated with narmafotinib (10 mg/kg) followed by skin flap surgery and imaging 24 hours post treatment. For skin flap surgery, mice were terminally anaesthetized using a mix of 10 mg/kg xylazine and 50 mg/kg zoletil and with additional maintenance of anesthesia (gaseous isoflurane 3%, O₂ 1 L/min, with continuous vacuum to remove excess isoflurane). Subcutaneous tumors were surgically exposed by a small incision around the tumor which was further expanded using blunt dissection to separate the epidermis and dermis from the peritoneal wall thereby creating a skin flap. The skin flap was expanded to a distance from the body suitable for intravital imaging. Once the tumors were surgically exposed, imaging was performed with mice restrained on a heated stage at 37°C and maintained under anesthesia, for a maximum of 40 minutes. Following intravital imaging tumors were formalin fixed for histological processing.

Subcutaneous Injections of PDCLs

[0113] 1.5×10^6 TKCC10lo cells in PBS/Matrigel (1:1) per mouse were injected into the rear flank of NOD.Cg-PrkdcscidIL2rgtm1Wjl/SzAusb mice whilst under anesthesia (isoflurane 3%, O₂ 1 L/min, with continuous vacuum to remove excess isoflurane). Tumors were allowed to develop for 23-29 days to an average volume of 50 mm³ before treatment schedules commenced. Treatment schedules began with twice daily oral gavage of narmafotinib (10

mg/kg) or vehicle (commencing Day 1: PM, finishing Day 4: AM) followed by in solution Oxaliplatin (Clifford Hallam Healthcare Pty Ltd, 5 mg/kg), Irinotecan (Clifford Hallam Healthcare Pty Ltd, 25 mg/kg), Calcium Leucovorin (Clifford Hallam Healthcare Pty Ltd 100 mg/kg) and 5-Fluorouracil (Baxter Healthcare Pty Ltd, 25 mg/kg) or saline vehicle were administered by intraperitoneal injection on Days 8 and 9, with the cycle recommencing after day 12 (Figure 6A). For timed endpoint studies, mice were treated with narafotinib (10 mg/kg) or vehicle twice daily for three days (commencing Day 1: PM and finishing Day 4: AM) over two cycles with the final treatment 4 hours prior to tumor collection for timed endpoint mice. In solution Oxaliplatin (Clifford Hallam Healthcare Pty Ltd, 5 mg/kg), Irinotecan (Clifford Hallam Healthcare Pty Ltd, 25 mg/kg), Calcium Leucovorin (Clifford Hallam Healthcare Pty Ltd 100 mg/kg) and 5-Fluorouracil (Baxter Healthcare Pty Ltd, 25 mg/kg) or saline vehicle were administered by intraperitoneal injection on Days 8 and 9, for two cycles with tumors collected 24 hours after the final treatment.

Orthotopic Injections

[0114] For orthotopic survival experiments NOD.Cg-PrkdcscidIL2rgtm1Wjl/SzAusb mice were anesthetized (isoflurane 3%, O₂ 1 L/min, with continuous vacuum to remove excess isoflurane) and TKCC10lo-Luc cells (1x10⁶ in 50 µl PBS/Matrigel (1:1) per mouse), were injected into the pancreas during open laparotomy (7). Briefly, a left subcostal incision was made through the skin and peritoneum exposing the pancreas, into which tumor cells were injected using a 29G needle. The peritoneal wall was then sutured using vicryl resorbable sutures and clipped. For analgesia, mice were treated subcutaneously with buprenorphine (0.075mg/kg) and topically with Bupivacaine (8 mg/kg). Treatment started when tumors were both palpable and visible by IVIS monitoring (average flux 1x10⁹). Treatment began with twice daily oral gavage of narafotinib (10 mg/kg) or vehicle (commencing Day 1: PM, finishing Day 4: AM) in solution Oxaliplatin (Clifford Hallam Healthcare Pty Ltd, 5 mg/kg), Irinotecan (Clifford Hallam Healthcare Pty Ltd, 25 mg/kg), Calcium Leucovorin (Clifford Hallam Healthcare Pty Ltd 100 mg/kg) and 5-Fluorouracil (Baxter Healthcare Pty Ltd, 25 mg/kg) or saline vehicle on Days 8 and 9 (Figure 7B). Tumor growth was monitored using IVIS imaging once per week. Experimental endpoint was determined upon development of ascites, overnight weight loss of ≥ 10% or total weight loss of ≥ 20% over the entirety of the experiment, hunching posture or signs of pain. At endpoint animals were euthanized and the pancreatic tumor, liver, lungs, and spleen were harvested, visible metastases were quantified, and tissues formalin fixed for histological processing. Mice were excluded from study as censored events if endpoint occurred due to non-PDAC related symptoms.

Immunoblot

[0115] Cells were rinsed twice in PBS and lysates prepared in RIPA protein lysis buffer (50 mM HEPES, 1% Triton X-100, 0.5% Sodium deoxycholate, 0.1% SDS, 0.5 mM EDTA, 50 mM NaF, 10 mM Na₃VO₄ and 1 x protease inhibitor cocktail (Roche)). Protein concentration was determined by Bradford assay and the lysate volumes were adjusted accordingly to a final concentration of 1 µg/µL. Protein separation was performed by gel electrophoresis using 4-12% or 10% Bis-Tis Protein Gels. Separated proteins were transferred onto PVDF membranes, which were blocked overnight at 4 °C in BSA dissolved in Tris-buffered saline, 0.1% Tween 20 (TBST). After rinsing with TBST membranes were incubated overnight at 4 °C in primary antibody solutions (TBS/BSA). Antibodies and their respective dilutions are provided in **Table 1**. After rinsing with TBST, membranes were then incubated with horseradish peroxidase (HRP)-linked secondary antibodies (1:5000, diluted in 1% skim milk/TBST, GE Healthcare Limited), for 2 hours at room temperature, and rinsed with TBST. Ultra-Enhanced Chemiluminescence (ECL) or ECL reagent were used to visualize HRP signal imaged on a Fusion FX (Vilber). Densitometry analysis of protein signal was performed in Image J (NIH).

Table 1. Primary antibodies utilized for immunoblotting.

Antibody	Company	Product	Dilution
Total FAK	BD Bioscience	610088	1:500
pTyr-397 FAK	Invitrogen	44-625G	1:500

[0116] For Picrosirius Red staining, the sections were dewaxed in xylene and rehydrated in graded ethanol washes. Following hematoxylin counterstaining sections were stained with 0.02% phosphomolybdic acid and 0.1% Picrosirius Red (Polysciences) for fibrillar collagen. Sections were then rinsed in acidified water and dehydrated in graded ethanol prior to cover slipping. Slides were scanned using an Aperio slide scanner. For APMA tumor sections, analysis of Picrosirius Red coverage was analyzed using Image J (see Macro: Picrosirius red Coverage), whilst an in-house MATLAB (Mathworks, US) script was used to analyze intensity and coverage of Picrosirius Red stained organotypic matrices and tumours.

[0117] For IHC on the Leica Bond RX, organotypic matrix and tumor sections were dewaxed using Bond Dewax Solution (Leica, AR2992) on a Leica Bond RX followed by heat-induced epitope retrieval (HIER) at 93 °C for organotypic matrix sections and 100 oC for tumor sections, with epitope retrieval solution 2 (pH=9, Leica AR9640) for 30 minutes, except for cleaved caspase-3 which was performed for 20 minutes. The details for primary antibody dilution and incubation time are provided in **Table 2**. IHC staining was carried out on the Leica Bond RX autostainer followed by hematoxylin counterstaining on a Leica Autostainer XL and cover

slipping on a Leica Coverslipper (CV5030). Samples were scanned using an Aperio slide scanner. Cleaved caspase-3 and Ki67 stained organotypic matrix and tumor sections were analyzed for positive and negative cells, and for pTyr-397-FAK (pTyr-397) DAB intensity was assessed using QuPath (P. Bankhead et al., *Sci Rep* 7, 16878 (2017))).

Table 2. Primary antibodies utilized for Immunohistochemistry, with staining performed on the Leica Bond RX Autostainer

Antibody	Company	Product	Dilution	Incubation (mins)
Ki67 (SP6)	Thermo Fisher Scientific	RM-9106-S1	1:500	60
Cleaved Caspase-3	Cell Signaling	9661	1:200	60
pTyr-397-FAK HUMAN TISSUE	Abcam	ab39967	1:4000	30
pTyr-397-FAK MOUSE TISSUE	Thermo Fisher Scientific	700255 (31H5L17)	1:4000	60

Imaging techniques and data analysis *in vitro* and *in vivo*

[0118] Polarized light imaging of Picrosirius Red staining: Polarized light microscopy was performed on fixed, deparaffinized and rehydrated 4 μm sections stained with 0.1% Picrosirius Red (Polysciences, 29401-250). Polarized light signal of fibrillar collagen was taken using an Olympus U-Pot polarizer and an Olympus U-ANT transmitted light analyzer fitted to a DM4000 microscope (Leica). Quantification of birefringent signal was analyzed using Image J. Briefly, Hue-Saturation Balance (HSB) thresholding was applied (high birefringence/red-orange $0 > H < 29$ | $0 > S < 255$ | $70 > B < 255$, medium birefringence/yellow $30 > H < 44$ | $0 > S < 255$ | $70 > B < 255$, low birefringence/green $45 > H < 245$ | $0 > S < 255$ | $70 > B < 255$). Relative area of fibers was then calculated as % of total fibers ($0 > H < 245$ | $0 > S < 255$ | $70 > B < 255$).

[0119] Second Harmonic Generation (SHG) imaging: SHG imaging was performed on an inverted Leica DMI 6000 SP8 confocal microscope with a Titanium-Sapphire femtosecond laser (Coherent Chameleon Ultra II) excitation source, operating at 80 MHz and tuned to a wavelength of 880 nm. SHG intensity was recorded on an RLD-HyD at 440/20 nm. For organotypic matrices, 3 representative fields of view (512 px x 512 px) were imaged over a 3D z-stack (80 μm depth with a 2.52 μm step size, and 30 μm depth with a 1.26 μm step size, respectively). For tissue sections, 5 regions of interest of deparaffinized, rehydrated 4 μm unstained sections were imaged with a step size of 1.26 μm and 20 μm depth. SHG signal intensity was quantified using MATLAB (Mathworks, US). For tumor samples from the APMA cohort, samples were tile scanned using a Leica Stellaris 8 DIVE multiphoton inverted

microscope. A Ti:Sapphire femtosecond pulsed laser (MaiTai eHP DeepSea, Spectra Physics) was used as excitation source, operating at 80 MHz, and tuned to a wavelength of 880 nm with SHG intensity recorded on an RLD-HyD at 440 nm. Individual z-stacks were then exported and SHG coverage analysis performed using Image J.

[0120] *FUCCI cell cycle reporter imaging:* Imaging of the FUCCI cell cycle reporter in live tissues was performed on an inverted Leica Stellaris 8 DIVE multiphoton inverted microscope. A Ti:Sapphire femtosecond pulsed laser (MaiTai eHP DeepSea, Spectra Physics) was used as excitation source, operating at 80 MHz and tuned to a wavelength of 920 nm with a 25x 0.95 NA water objective used for imaging. The signal was recorded using RLD-HyD detectors (using bandpass emission filters at 460/50 nm for SHG signal, 525/50 nm for mAzami Green and 585/40 for mKusabira Orange). Ten regions of interest (ROI, 512 px x 512 px) per tumor were imaged over a 20 μ m Z-stack with a step size of 2.52 μ m. 3D maximum projections were then generated using Leica LASX software and analyzed using QuPath to quantify percentage of red, green and yellow nuclei indicative of G1/0, G2/M or G1/S cell cycle phase, respectively.

[0121] *FLIM-FRET imaging of the FAK biosensor:* For in vitro measurements of FAK activity, 1×10^5 KPC-FAK cells were seeded onto CDMs and allowed to adhere, cells were then treated with vehicle or FAK inhibitor for 4 hours prior to FLIM-FRET imaging on CDMs. For in vivo measurements of ECFP fluorescence lifetime in subcutaneous xenografts, KPC-FAK cancer cells were injected into the flank of BALB/c-Fox1nuAusb mice. 4 hours post-treatment with vehicle or FAK inhibitor (10mg/kg) tumors were surgically exposed using skin flap surgery. Imaging was performed using an inverted Leica DMI 6000 SP8 confocal microscope with a Titanium-Sapphire femtosecond laser cavity (Coherent Chameleon Ultra II) excitation source tuned to a wavelength of 840 nm for ECFP excitation. Signal was recorded using RLD-HyD detectors (using bandpass emission filters at 435/40 nm for SHG signal, 483/40 nm for FLIM). FLIM data was acquired with a PicoHarp 300 TCSPC system (Picoquant) and image stabilization performed using Galene (S. C. Warren et al., Elife 7, (2018)). 50 cells per condition across multiple ROIs per tumor in vivo were acquired with a scan speed of 400 Hz, with 2 minutes 30 seconds acquisition time and a pixel dwell time of 5 μ s. Analysis of ECFP lifetimes was performed using FLIMfit by manual selection of single cell membrane regions of interest and recording of the exponential function fit to the fluorescence decay data. Reference lifetimes were calculated using a Chroma slide. Lifetime maps were generated from raw data with a smoothing by a 2x2 pixel kernel, and application of a standard rainbow look-up table, with blue indicating low ECFP fluorescence lifetime and green to yellow indicating high ECFP fluorescence lifetime. The background intensity threshold was set to the average background pixel value for each image to exclude areas of unspecific signal and is shown on the lifetime maps as black.

[0122] *IVIS imaging*: Orthotopic tumor growth was monitored via luciferase signal imaging on an IVIS Spectrum (PerkinElmer). Luciferin (150 mg/kg, Gold Biotechnology) was administered by intraperitoneal injection, 3 minutes prior to imaging. Anesthetized (isoflurane 2 L, O₂ 1 L/min, with continuous vacuum to remove excess O₂ and isoflurane) mice were placed on the IVIS stage, exposing the left flank and the signal was acquired with open filters and small binning. Tumor burden was determined based on total flux.

Example 3 - Treatment regimen

[0123] For *in vitro* work, the fibroblasts were TIFs (Telomerase Immortalised Fibroblasts) whilst the cancer cells were KPC cells (PDAC mouse cells).

[0124] TKCC10lo patient derived cells were injected into the rear flank of NOGIL2 mice for subcutaneous studies or orthotopically into the pancreas for orthotopic studies. The tumour was allowed to grow until palpable.

[0125] Treatment for subcutaneous studies or orthotopic studies followed the regimen described in **Figure 1A and B** respectively. A FAKi (10 mg/kg) according to the present disclosure or a vehicle was administered over four days according to the schedule described, with the first day of administration counted as day 1. On day 8, Oxaliplatin (5 mg/kg) was administered in the AM, followed by Irinotecan (25 mg/kg) administered in the PM. On day 9, Leucovorin calcium (100 mg/kg) was administered in the AM, followed by Fluorouracil (25 mg/kg) administered two hours post treatment with Leucovorin calcium. The mice were monitored until day 12. The regimen was repeated for up to 20 cycles, or until the experimental endpoint was reached. At the experimental endpoint the mice were sacrificed, the tumour harvested and analysed.

The example FAKi (The compound of Formula I – called AMP945 or narmafotinib) was given in a pulsed-dosing regimen in combination with FOLFIRINOX to mice models following the treatment regimen summarised in **Table 3** below.

Table 3. Summary of dosing regimen including vehicle and route of administration.

Treatment	Dose level (mg/kg)	Dosing solution (mg/mL)	Dosing vol (μL/g)	Route of administration	Dosing frequency and duration	Vehicle solution
Vehicle AMP945	-	-	5	Oral Gavage	Twice Daily x7-8 hours apart (7:30-8am-3:30pm) 6 times per cycle	0.5% w/v Hydroxypropylmethyl cellulose, 0.5% v/v benzyl alcohol, 0.4% v/v Tween 80
AMP945	10	2	5	Oral Gavage	Twice Daily x 7-8 hours apart (7:30-8am-3:30pm) 6 times per cycle	0.5% w/v Hydroxypropylmethylcellulose, 0.5% v/v benzyl alcohol, 0.4% v/v Tween 80
Vehicle (all FOLFIRINOX drugs)	-	-	10	Intraperitoneal	Once Daily Four time per cycle (matched to each below)	Saline
Oxaliplatin	5	0.5	10	Intraperitoneal	Once Daily AM (7:30-8:30am) Once per cycle	Saline
Irinotecan	25	2.5	10	Intraperitoneal	Once Daily PM (3-3:30 pm) Once per cycle	Saline
Leucovorin Calcium	100	10	10	Intraperitoneal	Once Daily AM (9-10am) Once per cycle	Saline
Fluorouracil (5-FU)	25	2.5	10	Intraperitoneal	Once Daily (11am-12pm) Once per cycle	Saline

[0126] Mouse survival was increased upon AMP945 priming compared to FOLFIRINOX alone (**Figure 2** – subcutaneous study). Treatment following subcutaneous cell injection shows that patient survival increases by roughly 35% following the combination of AMP945 and FOLFIRINOX.

[0127] An orthotopic patient-derived model was utilized to assess the long-term effect of AMP945 in combination with FOLFIRINOX on survival. Following orthotopic (intrapancratic) injection of TKCC10lo cells expressing luciferase into mice (**Figure 1B**), tumor growth was monitored *via* whole-body In Vivo Imaging System (IVIS) imaging. Upon reaching an average flux of 1×10^9 via IVIS imaging, mice were randomly assigned to separate groups receiving pulsed treatment cycles as described before consisting of 3 days priming with AMP945 prior to administration of FOLFIRINOX on Days 8 and 9 until study endpoint was reached.

[0128] AMP945 priming prior to FOLFIRINOX, provided a significant survival benefit over FOLFIRINOX alone demonstrating that AMP945 priming can improve FOLFIRINOX efficacy,

resulting in an increase in survival in this setting. In mice bearing patient-derived xenographs of pancreatic cancer, median survival is significantly extended when AMP945 is used in combination with a FOLFIRINOX chemotherapy (**Figure 3** and table below).

	Median survival
Vehicle/Saline	75.5 days
AMP945/saline	84 days
Vehicle/FOLFIRINOX	116 days
AMP945/ FOLFIRINOX	143 days

Example 4 – Tumour volume

[0129] Once control (vehicle or FOLFIRINOX only) mice reached the endpoint, matched AMP945 primed mice were also collected. Tumours were formalin fixed and paraffin embedded prior to IHC analysis.

[0130] The combination therapy shows that tumour volume grows at a slower rate when compared to FOLFIRINOX alone (**Figure 4A and B**). Tumour Volume (mm³) at endpoint (**Figure 4A**) and over time in days (**B**) for mice treated with FOLFIRINOX monotherapy or primed with AMP945 prior to FOLFIRINOX.

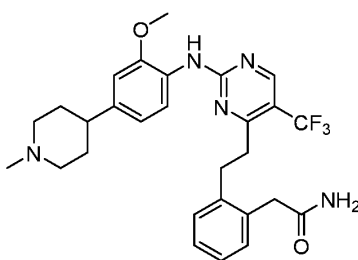
[0131] A decrease in tumour volume typically occurs in two ways: cell death or reduced cell proliferation. From a treatment perspective, reductions in tumour volume due to cell death is preferable to reduced proliferation.

[0132] Caspases are a family of enzymes crucial for initiating and executing apoptosis within a cell. Caspase-3 is a protein that is cleaved and thus activated upon the initiation of apoptosis. Increased levels in an assay are therefore indicative of increased apoptosis and can be utilised to investigate to evaluate the cytotoxic strength and effectiveness of potential therapeutic agents. **Figure 5A** (cleaved Caspase-3 positive cells (%)) demonstrates that the combination therapy resulted in higher increases in Caspase-3 cleavage compared to FOLFIRINOX alone. This is supportive of the administration of the FAKi AMP945 increasing the efficacy of FOLFIRINOX.

[0133] The expression of Ki67 is strongly associated with tumour cell proliferation and growth, and is widely used as a proliferation marker. It can be seen from **Figure 5B** that there is no significant difference between cell proliferation in subjects that received FOLFIRINOX alone compared to the combination therapy, indicating that the decrease in tumour volume is most likely due to apoptosis rather than a decrease in cell proliferation.

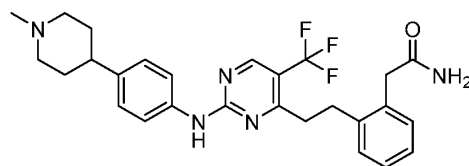
CLAIMS

1. A method of treating cancer in a subject in need thereof, the method comprising administering a combination of a FAK inhibitor and a FOLFIRINOX chemotherapy treatment regimen.
2. A combination of a FAK inhibitor and a FOLFIRINOX chemotherapy treatment regimen for use in the treatment of cancer in a patient in need thereof.
3. Use of a FAK inhibitor in the manufacture of a medicament for treating cancer in a patient in need thereof, by combination therapy employing the FAK inhibitor with a FOLFIRINOX chemotherapy treatment regimen.
4. The method, combination or use of any one of claims 1 to 3, wherein the FAK inhibitor is administered prior to the administration of the FOLFIRINOX chemotherapy treatment regimen.
5. The method, combination or use of claim 4 wherein administration of the FAK inhibitor is ceased prior to the administration of the FOLFIRINOX chemotherapy treatment regimen.
6. The method, combination or use of any one of claims 1 to 4, wherein the FAK inhibitor is administered in a continuous-dosing regimen.
7. The method, combination or use of any one of claims 1 to 6, wherein the FAK inhibitor defined by Formula (I) or (II) or a pharmaceutically acceptable derivative thereof:



Formula (I)

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Formula (II).

8. The method, combination or use of claim 7 wherein the FAK inhibitor is a compound of Formula (I).
9. The method, combination or use of claim 7 or claim 8, wherein the FAK inhibitor is a tartrate salt.
10. The method, combination or use of any one of claims 1 to 9, wherein the cancer is selected from a solid cancer, including but not limited to bone cancer, brain stem glioma, breast cancer, cancer of the adrenal gland, cancer of the anal region, cancer of the bladder, cancer of the endocrine system, cancer of the oesophagus, cancer of the head or neck, cancer of the kidney or ureter, cancer of the liver, cancer of the parathyroid gland, cancer of the penis, cancer of the small intestine, cancer of the thyroid gland, cancer of the urethra, carcinoma of the cervix, carcinoma of the endometrium, carcinoma of the fallopian tubes, carcinoma of the renal pelvis, carcinoma of the vagina, carcinoma of the vulva, colon cancer, cutaneous or intraocular melanoma, fibrosarcoma, lung cancer, lymphocytic lymphomas, neoplasms of the central nervous system (CNS), ovarian cancer, pancreatic cancer, pituitary adenoma, primary CNS lymphoma, prostate cancer, rectal cancer, renal cell carcinoma, sarcoma of soft tissue, skin cancer, spinal axis tumours, solitary fibrous tumour, stomach cancer and uterine cancer.
11. The method, combination or use of claim 10, wherein the cancer is pancreatic cancer, ovarian cancer, colon cancer, rectal cancer, fibrosarcoma or solitary fibrous tumours.