Title: POLYPEPTIDE COMPRISING THE SEQUENCE EGPWLEEEEEAYG LINKED TO AN IMMUNOGENIC CARRIER

Abstract: The present invention provides a compound comprising a polypeptide conjugated to an immunogenic carrier, the polypeptide comprising the sequence EGPWLEEEEEAYG (SEQ ID NO: 1) and having fewer than 17 amino acid residues, for use in medicine. Further, the invention provides a compound comprising a polypeptide conjugated to an immunogenic carrier, the polypeptide comprising the sequence EGPWLEEEEEAYG (SEQ ID NO: 1) and having fewer than 17 amino acid residues, wherein the immunogenic carrier is diptheria toxoid, tetanus toxoid, keyhole limpet haemocyanin, Purified protein derivative (PPD), Albumin, and/or viral proteins. The polypeptide may be conjugated to the immunogenic carrier via a cysteine residue. Pharmaceutical compositions and kits of parts are also provided. In particular, the use of the compounds in medicine and in the treatment of cancer is provided.
POLYPEPTIDE COMPRISING THE SEQUENCE EGPWLEEEEEAYG LINKED TO AN IMMUNOGENIC CARRIER

The present invention relates to compounds and medical uses. In particular, the invention relates to improved compounds for the generation of antibodies to gastrin 17 and uses thereof for the treatment of conditions, such as cancer.

Gastrin is a hormone that exists in a number of different forms including preprogastrin, progastrin, gastrin 34 (G34) and gastrin 17 (G17; SEQ ID NO: 6) as well as glycine-extended forms of at least the latter two (Dockray et al, 2001). Tumours of gut-derived tissues have been shown to respond trophically to gastrin 17 and glycine-extended gastrin 17 (Watson & Gilliam, 2001) with promotion of the growth of cancers of the colon, liver, stomach, oesophagus, thyroid medulla and lung (Watson, Durrant 1989; Koh 1996 & 2004; Goetze 2000). Elimination of gastrin 17 by immunological means is potentially more useful than inhibition of the hormone at its receptor by synthetic antagonists since there appear to be multiple receptors responsible, at least in part, for trophic activity of gastrin 17 and glycine-extended gastrin 17 (Stepan 1996 & 1999; Koh 1996) and therefore, it may not be possible to prepare synthetic antagonists to all such receptors.

Antibodies to the N-terminus of gastrin 17 have been shown to be useful in counteracting the trophic effects of gastrin 17 in animal models of human cancers (Watson & Gilliam, 2001). One animal model has shown utility of this immunological approach in reducing growth of a colonic tumour, DHDK12, a cell line derived from a rat colon cancer, using a rat-specific gastrin immunogen for active immunisation (Watson, Michaeli, Grimes et al, 1996)

Active immunization of patients with pancreatic, colorectal and gastric cancers with gastrin 17 immunogens has been investigated in several clinical trials. This therapy has been shown to provide benefit to pancreatic cancer patients when used as the sole treatment for their disease (Gilliam et al. 2004; Takhar et al 2006). The immunogen used in this clinical trial was a conjugate of a peptide representing the N-terminal 9 amino acids of gastrin 17 and diphtheria toxoid (DT). A spacer peptide of SSPP PPC (SEQ ID NO: 2) was added to the C-terminus of the gastrin 17 N-terminus 9-mer peptide which permitted it to be linked to the lysine residues on diphtheria toxoid using a bifunctional compound that couples the cysteine on the peptide to the free amino groups of the DT lysines (US 5,468,494).
Similar immunogens using the 9 N-terminal amino acids of gastrin 17 but with a different spacing peptide, GGGGSC (SEQ ID NO: 3) bound to either mutant DT (CRM 197) as carrier protein, or to P64K from the outer membrane of Neisseria meningitidis, have also been shown to be effective in raising antibodies to the N terminus of gastrin 17 that were inhibitory to tumour growth in an in vitro model (Xiang-Hua Xiong et al 2006). Later efforts by the same investigators using a recombinant immunogen, produced lower levels of anti-gastrin antibodies (Xiang-Hua Xiong et al 2006).

Use of another peptide spacer, RPPPPC (SEQ ID NO: 4), was described in US 5,023,077 in combination with several smaller N-terminal fragments (up to 8 amino acids) of gastrin 17 peptide conjugated to diphtheria toxoid as being effective in suppressing human gastrin 17-induced acid secretion in an anaesthetised rat model. US 5,607,676 describes anti-tumour activity of antibodies raised from lengths of 4 to 6 amino acids of the N-terminus of gastrin 17 coupled with the spacing peptide, RPPPPC (SEQ ID NO: 4) to diphtheria toxoid (DT). US 5,607,676 also describes sequences of the gastrin 17 N terminus of 10, 11 and 12 amino acids in length: no data is provided in relation to these longer sequences.

US 6,132,720 describes both of the spacer sequences SEQ ID NOs: 3 and 4 as providing a critical contribution to the immunogenicity of another conjugated peptide immunogen - Gonadotrophin releasing hormone, GnRH, coupled via either spacer peptide to diphtheria toxoid.

It has previously been taught that the presence of either of the two spacing peptides, SEQ ID NOs: 3 or 4, is necessary to provide adequate immunogenicity. It has been suggested that the spacers improve immunogenicity by projecting the B-cell epitope out towards surrounding lymphoid cells, thus presenting more prominently the relevant amino acid sequence for the detection by host lymphocytes and antigen presenting cells. This outward extension is achieved by use of amino acid sequences designed to give linearity and rigidity of stereochemical structure such as those described in US 5,468,494; US 5,023,077; and US 5,622,702.

EP 0 804 737 describes the use of gastrin-17 fragment 1-13 coupled to rabbit autologous red blood cells to raise polyclonal antibodies to the G17 peptide for use in research.
The listing or discussion of an apparently prior-published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge.

The present inventors have surprisingly found that compounds that lack the spacer polypeptides comprised in the peptides previously described promoted a greater immune response.

Thus, in a first aspect the present invention provides a compound comprising a polypeptide conjugated to an immunogenic carrier, the polypeptide comprising the sequence EGPWLEEEEEAYG (SEQ ID NO: 1) and having fewer than 17 amino acid residues, for use in medicine.

The novel compounds of the present invention comprises the sequence EGPWLEEEEEAYG (SEQ ID NO: 1), which corresponds with the N-terminal 13 amino acids of gastrin 17. The compounds of the present invention do not comprise a heterologous spacer peptide between the gastrin epitope and the immunogenic carrier molecule. The art suggests that such a spacer is necessary but according to the surprising results provided herein, a heterologous spacer is not necessary in order to promote a strong immune response thus obtain high levels of antibodies. The data provided herein is thus contrary to the prejudice in the art. The compounds of the invention may act as effective vaccines for the treatment or prevention of diseases involving gastrin 17 and/or its N-terminal fragments, such as cancer.

It is envisaged that when administered parentally to a mammal in an appropriate vehicle, the compounds of the present invention will be capable of eliciting in that mammal, antibodies reactive with human Gastrin 17 and/or antibodies capable of neutralizing the biological activity of human Gastrin.

Not wishing to be bound by any theory, it is postulated here that the increased length of the N-terminus fragment / sequence of gastrin 17 comprised in the compound of the invention over the compounds provided previously (for example compounds consisting of the 9 N-terminal G17 amino acids conjugated to DT via a spacer) and the lack of a heterologous spacer peptide in the present compounds may contribute to higher antibody titres by not distorting the conformation of the G17 N-terminus epitope, by exposing a larger proportion of the gastrin 17 molecule to B-cell lymphocytes, especially the proximal part of the sequence which forms the central part of the complete gastrin 17
peptide and/or by eliminating the potential for production of antibodies reactive with a heterologous spacer rather than gastrin 17. Such antibodies have been invoked to explain unexpected and confusing results in assays described in earlier patents, (see US 5607676, column 11, line 45). Further, He et al (2005) Am J Physiol Gastroin test Liver Physiol 289:G478-G488 and Pannequin et al (2002) JBC 277: 48602-9 have suggested that the stability of the tertiary structure of Gastrin 17 is aided by interactions of the side chains of Tyr at position 12 with Leu at position 5. Conjugates provided in the prior art do not contain Tyr 12. The additional stability and tertiary structure provided by the inclusion of both Leu 5 and Tyr 12 in the compositions of the present invention may contribute to the production of higher affinity antibodies than those produced using conjugates provided in the prior art.

By "conjugated" we include the meaning of the formation of any covalent linkage between two or more molecules. Thus, the term "conjugated" may be used interchangeably with the terms "covalently bound", "attached", or any other term that indicates a chemical coupling.

By "immunogenic carrier" we include the meaning of any molecule capable of promoting an immune response in an individual, when introduced into that individual. Examples of such carriers will be well known to those skilled in the art and include diphtheria toxoid, tetanus toxoid, keyhole limpet haemocyanin, Purified protein derivative (PPD), Albumin, and/or viral proteins.

By "capable of promoting an immune response" we include the meaning that the immunogenic carrier acts as an adjuvant. Thus, when introduced into the circulation of the individual, the immunogenic carrier is able to stimulate a humoral and/or cell-based immune response in the individual leading to the production of antibodies and/or T-lymphocytes specific to the immunogenic carrier but also to the production of antibodies and/or T-lymphocytes specific to the polypeptide of the invention as it is covalently linked to the polypeptide of the invention, as would be understood by a person of skill in the art.

The polypeptide of the invention may be synthetically produced, for example by solid phase synthesis, or it may be expressed in an appropriate gene expression system or it may be purified from a natural source, as appropriate.

In an embodiment, the compound for use in medicine according to the first aspect may be for use in treating cancer.
It is envisaged that in an embodiment the immunogenic carrier of the compounds of the invention may be selected from, but not limited to, the group comprising diphtheria toxoid, tetanus toxoid, keyhole limpet haemocyanin, Purified protein derivative (PPD), Albumin, and/or viral proteins.

In a second aspect, the present invention provides a compound comprising a polypeptide conjugated to an immunogenic carrier, the polypeptide comprising the sequence EGPWLEEEAYG (SEQ ID NO: 1) and having fewer than 17 amino acid residues, wherein the immunogenic carrier is diphtheria toxoid, tetanus toxoid, or keyhole limpet haemocyanin.

In an embodiment of any aspect of the invention, the polypeptide may consist of the sequence EGPWLEEEAYG (SEQ ID NO: 1). Thus, the polypeptide in this embodiment will consist of the 13 N-terminal amino acids of gastrin 17 conjugated to an immunogenic carrier.

In a further embodiment of any aspect of the invention, the polypeptide may be conjugated to the immunogenic carrier via a unique residue. The unique residue may be, for example, a cysteine or a lysine residue. The cysteine (or lysine) residue is a unique residue that is used to bind to a coupling agent/heterobifunctional molecule (Bioconjugation Dent A, 1998 Macmillan/Stockton Press). (See www.pbcpeptide.com/conjugation describing cys or lys linking of peptides and proteins or US patent 7807778 for many references to this technique). The conjugation to the immunogenic carrier may be site-specific. In an embodiment, the coupling moiety may comprise eMCS - epsilon maleimido caproic acid N-hydroxy succinimide ester - which will bind the cysteine residue. There are other similar bifunctional linkers / cross-linking reagents in common use that may be used in the compounds of the present invention, for example, GMBS (Chemical Name: N-(4-Maleimidobutryloxy)succinimide), HMCS (Chemical Name: N-(8-Maleimidocapryloxy)succinimide), KMUS (Chemical Name: N-(1-Maleimidoundecanoyloxy)succinimide), SPDP (Chemical Name: N-Succinimidyl 3-(2-pyridyldithio)propionate), Sulfo-EMCS (Chemical Name: N-(6-Maleimidocaproyloxy)sulfosuccinimide), Sulfo-GMBS (Chemical Name: N-(4-Maleimidocaproyloxy)sulfosuccinimide), Sulfo-HMCS (Chemical Name: N-(8-Maleimidocaproyloxy)sulfosuccinimide), and/or Sulfo-KMUS (Chemical Name: N-(11-Maleimidoundecanoyloxy)sulfosuccinimide).
While the compounds of the invention may comprise a linking amino acid and/or a coupling agent in certain embodiments, it is intended that they do not contain a spacer, e.g. spacing peptide or other molecule. The inventors have surprisingly found, contrary to popular belief in the art, that a spacer is not necessary and in fact the absence of a spacer provides better antibody responses.

By "spacer" we include the meaning of a polypeptide heterologous to the polypeptide of the compounds of the invention that acts to distance the polypeptide of the invention from the immunogenic carrier. A spacer may for example be a polypeptide consisting of 3 or more amino acids, for example 3 to 10 amino acids. The spacer may alternatively comprise variants, derivatives or fragments of the amino acids of equivalent length, or non-peptide mimetics, or other non-peptide molecules that act to distance the moieties of the invention from one another, for example a carbohydrate and/or lipid molecule. It is envisaged that the spacer will be of a size that is significant in comparison with the size of the polypeptide of the compounds of the invention. A linking molecule such as the unique residue (e.g cysteine) and/or a coupling agent/heterobifunctional molecule is not a spacer within the definition provided herein.

It is preferred that the immunogenic carrier is conjugated at the C-terminal end of the polypeptide. Nevertheless, if appropriate then the immunogenic carrier may be conjugated at the N-terminal end of the polypeptide, as would be understood by the person of skill in the art. The techniques used for conjugation are well known and an example of which is provided in Example 1.

In a third aspect, the present invention provides a pharmaceutical composition/formulation comprising the compound of the second aspect and a pharmaceutically acceptable excipient, adjuvant, diluent or carrier.

Preferably, the composition/formulation is a unit dosage containing a daily dose or unit, daily sub-dose or an appropriate fraction thereof, of the active ingredients.

The composition of the invention will normally be administered by any parenteral route, in the form of a pharmaceutical formulation comprising the active ingredients, in a pharmaceutically acceptable dosage form. Depending upon the disorder and patient to be treated, as well as the route of administration, the compositions may be administered at varying doses.
The composition of the invention can be administered alone but will generally be administered in admixture with a suitable pharmaceutical excipient, diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice.

Thus, the composition of the invention can also be administered parenterally, for example, intravenously, intra-arterially, intraperitoneally, intrathecal\(^n\), intraventricularly, intracranially, intra-muscularly or subcutaneously, or may be administered by infusion techniques. The compositions are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well-known to those skilled in the art.

Thus, in an embodiment of the third aspect, the composition/formulation may be formulated for parenteral delivery.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, 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. The formulations may be presented in unit-dose or multi-dose containers, for example sealed 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 of the kind previously described.

For parenteral administration to human patients, the daily dosage level of the compounds of the invention, or compounds for the medical uses of the invention, will usually be from 0.01 to 10 mg per adult \(i.e.\) from about 0.00015 to 0.15 mg/kg, administered in single or divided doses. It is envisaged that an appropriate dose would be 250 or 500 or 1000 meg per injection repeated in a schedule of 0, 1, 3, 6, 12, 18, 24 etc weeks.

The physician in any event will determine the actual dosage which will be most suitable for any individual patient and it will vary with the age, weight and response of the
particular patient. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited and such are within the scope of this invention.

The present invention also provides the compound or pharmaceutical composition/formulation of the invention for use in medicine. In particular, the present invention provides the compound or pharmaceutical composition/formulation of the invention for use in treating cancer.

In an embodiment, the cancer to be treated may be a cancer of the gastrointestinal tract. The cancer to be treated by the compounds/compositions of the invention may be pancreatic cancer, colorectal cancer, gastric cancer, stomach cancer, biliary tract cancer and/or oesophageal cancer. Further, the cancer to be treated by the compounds/compositions of the invention may be lung cancer, thyroid cancer and/or medullary thyroid cancer. The compounds/pharmaceutical compositions/formulations of the invention may also be useful in treating other cancers with Gastrin involvement, as would be understood by a person of skill in the art.

In an embodiment the pharmaceutical compositions/formulations of the invention may further comprise one or more further anticancer agents, anti-cancer compositions, and/or cytotoxic compounds.

It is envisaged that dual therapy/therapy with multiple agents including the compounds/compositions of the invention may lead to more efficacious treatment of the patient through additive or synergistic effects of the compounds with one another. Therapy with multiple compounds/compositions as active ingredients may help to reduce or eliminate any potential resistance that may develop in the patient to the effects of any one or more of the active ingredients during therapy.

It is envisaged that the one or more further anticancer agents, anti-cancer compositions, and/or cytotoxic compounds may be for administration to the patient before, at the same time as, or after administration of the pharmaceutical compositions/formulations of the invention.

The compounds/compositions of the invention may be co-formulated with the further anti-cancer agents, anti-cancer compositions, and/or cytotoxic compounds, or the
compounds/compositions of the invention may be formulated separately, as appropriate as would be understood by a person of skill in the art.

In a fourth aspect, the present invention provides a kit of parts comprising: i) a compound or pharmaceutical composition/formulation according to the second or third aspects of the invention; and ii) one or more further anticancer agents, anti-cancer compositions, and/or cytotoxic compounds.

In any embodiment of the compound, pharmaceutical composition/formulation, or kit of parts of any preceding aspect or embodiment, the compound, pharmaceutical composition/formulation or kit of parts may be for administration to a patient who is or has been treated with a further one or more anti-cancer agents, anti-cancer compositions, anti-cancer treatments and/or cytotoxic compounds. Thus, the patient may be administered the compound/composition of the invention after treatment of the patient with the further one or more anti-cancer agents, anti-cancer compositions, anti-cancer treatments and/or cytotoxic compounds, or during treatment with the further one or more anti-cancer agents, anti-cancer compositions, anti-cancer treatments and/or cytotoxic compounds.

In an embodiment of the compound, pharmaceutical composition or kit of parts of any preceding aspect or embodiment, the compound, pharmaceutical composition/formulation or kit of parts may be for sequential, or simultaneous administration with the further one or more anti-cancer agents, anti-cancer compositions, anti-cancer treatments and/or cytotoxic compounds.

The compound/composition of the invention may be for administration at the same time as the further one or more anti-cancer agents, anti-cancer compositions, anti-cancer treatments and/or cytotoxic compounds or before the further one or more anti-cancer agents, anti-cancer compositions, anti-cancer treatments and/or cytotoxic compounds.

Thus, the further one or more anti-cancer agents, anti-cancer compositions, anti-cancer treatments and/or cytotoxic compounds may be administered or carried out after the compound, pharmaceutical composition or kit of parts of the invention.

It is envisaged that the course of administration and timing of administration would be determined by a physician prior to therapy, as appropriate. Appropriate dosages will change according to patient characteristics and disease severity and would be determined by the physician as appropriate.
It is envisaged that the further anticancer agent, anti-cancer composition, or cytotoxic compound may be selected from, but not limited to, the group comprising an alkylating agent (ATC code L01a), an antimetabolite (ATC code L01b), a plant alkaloid or other natural product (ATC code L01c), a cytotoxic antibiotic or a related substance (ATC code L01d), or another antineoplastic agent, such as an anti-cancer antibody or vaccine.

In an embodiment, the antineoplastic agent is an alkylating agent (ATC code L01a), an antimetabolite (ATC code L01b), a plant alkaloid or other natural product (ATC code L01c), a cytotoxic antibiotic or a related substance (ATC code L01d), or another antineoplastic agent.

Hence, in an embodiment the antineoplastic agent is an alkylating agent selected from, but not limited to, the group comprising a nitrogen mustard analogue (for example cyclophosphamide, chlorambucil, melphalan, chlormethine, ifosfamide, trofosfamide, prednimustine or bendamustine), an alkyl sulfonate (for example busulfan, treosulfan, or mannosulfan), an ethylene imine (for example thiotepa, triaziquone or carboquone), a nitrosoareua (for example carmustine, lomustine, semustine, streptozocin, fotemustine, nimustine or ranimustine), an epoxide (for example etoglucid), and another alkylating agent (ATC code L01ax, for example mitobronitol, pipobroman, temozolomide or dacarbazine).

In another embodiment the antineoplastic agent is an antimetabolite selected from, but not limited to, the group comprising a folic acid analogue (for example methotrexate, raltitrexed, pemetrexed or pralatrexate), a purine analogue (for example mercaptopurine, tioguanine, cladribine, fludarabine, clofarabine or nelarabine), and a pyrimidine analogue (for example cytarabine, fluorouracil, tegafur, carmofur, gemcitabine, capecitabine, azacitidine or decitabine).

In another embodiment the antineoplastic agent is a plant alkaloid or other natural product selected from, but not limited to, the group comprising a vinca alkaloid or a vinca alkaloid analogue (for example vinblastine, vincristine, vindesine, vinorelbine or vinflunine), a podophyllotoxin derivative (for example etoposide or teniposide), a colchicine derivative (for example demecolcine), a taxane (for example paclitaxel, docetaxel or paclitaxel poliglumex), and another plant alkaloid or natural product (ATC code L01cx, for example trabectedin).
In another embodiment the antineoplastic agent is a cytotoxic antibiotic or related substance selected from, but not limited to, the group comprising an actinomycin (for example dactinomycin), an anthracycline or related substance (for example doxorubicin, daunorubicin, epirubicin, aclacinomycin, zorubicin, idarubicin, mitoxantrone, pirarubicin, valrubicin, amrubicin or pixantrone), and another (ATC code L01dc, for example bleomycin, plicamycin, mitomycin or ixabepilone).

In a further embodiment the antineoplastic agent is another antineoplastic agent selected from, but not limited to, the group comprising a platinum compound (for example cisplatin, carboplatin, oxaliplatin, satraplatin or polyplatilien), a methylhydrazine (for example procarbazine), a monoclonal antibody (for example edrecolomab, rituximab, trastuzumab, alemtuzumab, gemtuzumab, cetuximab, bevacizumab, panitumumab, catumaxomab or ofatumumab), a sensitizer used in photodynamic/radiation therapy (for example porfimer sodium, methyl aminolevulinate, aminolevulinic acid, temoporfin or efaproxiral), and a protein kinase inhibitor (for example imatinib, gefitinib, erlotinib, sunitinib, sorafenib, dasatinib, lapatinib, nilotinib, temsirolimus, everolimus, pazopanib, vandetanib, afatinib, masitinib or toceranib).

In a still further embodiment the antineoplastic agent is another antineoplastic agent selected from, but not limited to, the group comprising amsacrine, asparaginase, altretamine, hydroxycarbamide, lonidamine, pentostatin, miltefosine, masoprocol, estramustine, tretinoin, mitoguazone, topotecan, tiazofurine, irinotecan, alitretinoin, mitotane, pegasparagase, bexarotene, arsenic trioxide, denileukin diftitox, bortezomib, celecoxib, anagrelide, oblimersen, sitimagene ceradenovec, vorinostat, romidepsin, omacetaxine mepesuccinate and eribulin.

It is envisaged that the further anticancer treatment may be selected from, but not limited to, the group comprising, radiotherapy and photodynamic therapy.

The invention further provides for the use of a compound or pharmaceutical composition/formulation or kit of parts according to any preceding aspect and embodiment in the manufacture of a medicament for treating cancer in a patient in need thereof.

Further, the invention provides a method of treating cancer comprising administering a compound, pharmaceutical composition/formulation or kit of parts according to any
preceding aspect and embodiment to a patient in need thereof. The cancer may be any cancer described above or any other suitable cancer.

It is envisaged that the patient in any aspect and embodiment of the invention will be a mammal. Such mammal may be selected from, but not limited to, the group comprising a human, a mouse, a rat, a hamster, a rabbit, a cat, a dog, a goat, a sheep, a monkey, an ape, or other appropriate laboratory, sporting or companion mammal. Preferably, the patient is a human.

In a further aspect, the invention provides a compound comprising a peptide comprising the N-terminal 13 amino acids of human gastrin 17 conjugated to an immunogenic carrier, wherein the compound is capable of eliciting antibodies reactive with human Gastrin 17 and/or neutralizing the biological activity of human Gastrin when administered in an appropriate vehicle, parentally to a mammal.

In an embodiment of any aspect of the present invention the peptide may be a synthetic peptide. In an embodiment, the synthetic peptide may be pyroEGPWLEEEEAYG. By "pyroE", we intend the meaning of pyro glutamic acid, as would be understood by a person of skill in the art.

In an embodiment of any aspect of the invention, the compound may be an analog compound wherein the synthetic peptide is pyroEGPWLEEEEAYGC.

In any aspect of the invention it is envisaged that the carrier and the peptide may be conjugated using the bifunctional reagent (N-(e-maleimidocaproyloxy) succinimide ester), known as eMCS. It is preferred that the molecular ratio of peptide to carrier is 8-20. It is particularly preferred that the ratio is 15.

In an embodiment of any aspect, the vehicle may be an emulsion of oil in water. It is preferred that the oil is mineral oil, squalane, squalene or a mixture of these and the water is a buffered saline solution. The emulsion may contain surfactants, stabilizers and/or thinning agents.

All documents referred to herein are hereby incorporated by reference.
The invention is now described in more detail by reference to the following, non-limiting, Figures and Examples.

EXAMPLE 1: Preparation of synthetic peptide DT conjugate (G17CDT)

The peptide of SEQ ID NO: 1, with a cysteine residue for conjugation, was synthesised by standard solid phase synthesis methods and was characterised as to amino acid content and purity. To couple the resultant peptide (14 amino acids) to diphtheria toxoid (DT), the eMCS conjugation method was used.

10 mg of DT was suspended in activation buffer (0.1 M sodium phosphate (pH = 7.0) + 0.1 mM EDTA) to 1 ml and stirred until dissolved. The DT was then activated by slow addition of 20 µl of the eMCS solution (10 mg of eMCS dissolved in 0.2 ml DMF, 50 mg/ml) under darkened conditions. The activated DT (DT-act) was purified using a centrifugal concentrator against conjugation buffer (0.1 M sodium phosphate buffer (pH = 6.5) + 0.1 mM EDTA) until the volume was reduced to 0.5 ml. This step was repeated three more times following which conjugation buffer was added to bring the total volume to 1.0 ml. Following transfer of the DT-act solution to a glass vial, the peptide was coupled to the DT-act by adding 5.0 mg peptide dissolved in 0.25 ml conjugation buffer while stirring under nitrogen in a capped vial at room temperature overnight in the dark. The resultant peptide-DT conjugate was purified in a centrifugal concentrator against 12 ml of PBS (phosphate buffered saline, pH = 7.2), reducing the volume to 0.5 ml; this step was repeated three more times. After the 4th centrifugation, PBS was added to bring the total volume to 2.0 ml. Protein concentration was confirmed by Lowry Assay against BSA standard and the peptide-DT solution was stored in a glass vial frozen at -20 °C.

The substitution ratio of the peptide was calculated from amino acid analysis of the conjugate and the DT and found to be about 15 moles of peptide per 100,000 MW of DT. This was assessed appropriate for immunogenicity studies.

EXAMPLE 2: Immunopharmacology of G17CDT

The peptide-DT conjugate (G17CDT; SEQ ID NO: 1 Cysteine DT conjugate) was formulated using Freund’s adjuvant (Complete for the first dose, Incomplete for subsequent doses). The product was administered to rabbits, using a 0, 3, 6 week dosing schedule, as a series of three or four multi-site subcutaneous injections. A dose
of 500 meg was used in the primary immunisation and 200 meg in the subsequent two immunisations.

Sera from blood collections on weeks six, eight and nine from the first immunisation were assayed for antibodies reactive to the free unconjugated peptide (G17(13)C; SEQ ID NO: 5) and to the full length human gastrin 17 molecule (commercially available: Bachem catalogue no. H 3805) by ELISA. Standard ELISA methodology was employed using positive and negative control sera with serial dilution until the signal was extinguished. The maximum titre was thus determined.

Mean maximum titres obtained to both the peptide immunomimic fragment (SEQ ID NO: 5), and to native gastrin 17 (SEQ ID NO: 6) are displayed in Table 1.

| TABLE 1 |
|-----------------|----------|----------|----------|----------|
| Mean titres...at| Week 0   | Week 6   | Week 8   | Week 9   |
| to:             |          |          |          |          |
| Gastrin 17      | <50      | 682,000  | 682,900  | 904,150  |
| G17(13)Cys      | <50      | 661,300  | 3,485,750| 3,576,150|

These results confirm the immunogenicity of the novel conjugate G17CDT and the capacity of the resultant antibodies to bind both the N-terminus 13 amino acid fragment of gastrin 17, the immunising antigen, as well as the full length natural human G17 molecule. Surprisingly, the potency of the conjugate in rabbits substantially exceeds that for conjugates made with spacer peptides.

By way of comparison, reference is made to literature data on antibodies generated by similar doses of the G17(9)DT immunogen in several studies in humans. Antibody titres shown to be effective in prolonging survival were >1200 (1 unit = titre/1,000) when the immunogen was given to pancreatic cancer patients as sole therapy (Gilliam et al. 2004) and 8,000 - 16,000 when used at higher doses in combination with gemcitabine (Shapiro et al. 2005).

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CLAIMS

1. A compound comprising a polypeptide conjugated to an immunogenic carrier, the polypeptide comprising the sequence EGPWLEEEEAYG (SEQ ID NO: 1) and having fewer than 17 amino acid residues, for use in medicine.

2. The compound of Claim 1, for use in treating cancer.

3. The compound Claim 1 or 2, wherein the immunogenic carrier is diphtheria toxoid, tetanus toxoid, keyhole limpet haemocyanin, Purified protein derivative (PPD), Albumin, and/or viral proteins.

4. A compound comprising a polypeptide conjugated to an immunogenic carrier, the polypeptide comprising the sequence EGPWLEEEEAYG (SEQ ID NO: 1) and having fewer than 17 amino acid residues, wherein the immunogenic carrier is diphtheria toxoid, tetanus toxoid, or keyhole limpet haemocyanin.

5. The compound of any of Claims 1 to 4, wherein the polypeptide consists of the sequence EGPWLEEEEAYG (SEQ ID NO: 1).

6. The compound of any of the preceding claims, wherein the polypeptide is conjugated to the immunogenic carrier via a cysteine residue, and/or eMCS, and/or GMBS, and/or HMCS.

7. The compound of any of the preceding claims, wherein the immunogenic carrier is conjugated at the C-terminal end of the polypeptide.

8. A pharmaceutical composition/formulation comprising the compound of any of Claims 4 to 7, and a pharmaceutically acceptable excipient, adjuvant, diluent or carrier.

9. The pharmaceutical composition/formulation of Claim 8, wherein the composition is formulated for parenteral delivery.

10. The compound or pharmaceutical composition/formulation of any of Claims 4 to 9, for use in medicine.
11. The compound or pharmaceutical composition/formulation of any of Claims 4 to 10, for use in treating cancer.

12. The compound or pharmaceutical composition/formulation of any of Claims 2, 3, or 11, wherein the cancer is a cancer of the gastrointestinal tract.

13. The compound or pharmaceutical composition/formulation of any of Claims 2, 3, 11 or 12, wherein the cancer is pancreatic cancer, colorectal cancer, stomach cancer, biliary tract cancer, gastric cancer or oesophageal cancer.

14. The compound or pharmaceutical composition/formulation of any of Claims 2, 3, or 11, wherein the cancer is lung cancer, thyroid cancer or medullary thyroid cancer.

15. The pharmaceutical composition/formulation of any of Claims 8 to 14, further comprising one or more further anticancer agents, anti-cancer compositions, and/or cytotoxic compounds.

16. A kit of parts comprising:
   i) a compound or pharmaceutical composition/formulation according to any of Claims 4 to 15; and
   ii) one or more further anticancer agents, anti-cancer compositions, and/or cytotoxic compounds.

17. The compound, pharmaceutical composition/formulation or kit of parts of any of Claims 1, 2, 3, or 8 to 16, wherein the compound, pharmaceutical composition or kit of parts is for administration to a patient who is or has been treated with a further one or more anti-cancer agents, anti-cancer compositions, anti-cancer treatments and/or cytotoxic compounds.

18. The compound, pharmaceutical composition/formulation or kit of parts of Claim 17, wherein the compound, pharmaceutical composition or kit of parts is for sequential, or simultaneous administration with the further one or more anti-cancer agents, anti-cancer compositions, anti-cancer treatments and/or cytotoxic compounds.

19. The compound, pharmaceutical composition/formulation or kit of parts of any of Claims 1, 2, 3, or 8 to 16, wherein the compound, pharmaceutical composition or kit of parts is for administration to a patient before the patient is treated with a further one or
more anti-cancer agents, anti-cancer compositions, anti-cancer treatments and/or cytotoxic compounds.

20. The composition, pharmaceutical composition/formulation or kit of parts of any of Claims 15 to 19, wherein the further anticancer agent, anti-cancer composition, or cytotoxic compound is an alkylating agent (ATC code L01a), an antimetabolite (ATC code L01b), a plant alkaloid or other natural product (ATC code L01c), a cytotoxic antibiotic or a related substance (ATC code L01d), or another antineoplastic agent, such as an anti-cancer antibody.

21. The composition, pharmaceutical composition/formulation or kit of parts of Claims 17 to 19, wherein the further anticancer treatment is radiotherapy and/or photodynamic therapy.

22. Use of a compound or pharmaceutical composition/formulation or kit of parts according to any preceding claim in the manufacture of a medicament for treating cancer in a patient in need thereof.

23. A method of treating cancer comprising administering a compound, pharmaceutical composition/formulation or kit of parts according to any of Claims 1 to 21 to a patient in need thereof.

24. The compound, pharmaceutical composition/formulation, kit of parts, use or method according to any preceding claim, wherein the patient is a human.

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INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K47/48 C07K7/08 C07K14/595 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database consulted during the international search (name of database and, where practicable, search terms used)

EPO-Internal , BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C. X See patent family annex.

* Special categories of cited documents:
   * "A" document defining the general state of the art which is not considered to be of particular relevance
   * "E" earlier application or patent but published on or after the international filing date
   * "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
   * "O" document referring to an oral disclosure, use, exhibition or other means
   * "P" document published prior to the international filing date but later than the priority date claimed
   * "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
   * "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search 6 February 2013

Date of mailing of the international search report 13/02/2013

Name and mailing address of the ISA/
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016

Authorized officer Bl i e m, Barbara

Form PCT/ISA210 (second sheet) (April 2005)
1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)
      - [ ] on paper
      - [ ] in electronic form

   b. (time)
      - [x] in the international application as filed
      - [ ] together with the international application in electronic form
      - [ ] subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

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