

Treatment of cancers associated with  $\beta$ -cateninField

The present disclosure and invention relate to the treatment of cancers associated with an aberrant Wnt/ $\beta$ -catenin signalling pathway. Specifically, we have discovered that an oligopeptidic compound (CyPep-1), previously shown to preferentially target and disrupt the plasma membrane of cancer cells, also acts by inhibiting the Wnt/ $\beta$ -catenin pathway. This dual therapeutic effect is of particular therapeutic interest since the use of CyPep-1 may affect, in particular, cancers that display aberrant activation of the Wnt/ $\beta$ -catenin signalling pathway. This compound has a distinct dual mechanism of action promoting immune activation and reversing immune exclusion. Also provided are methods to identify patients suitable for treatment of such cancers with the oligopeptidic compound.

Background

Despite recent advances in cancer treatment, cancer remains a significant cause of morbidity and mortality worldwide. In 2020, approximately 19.3 million patients were diagnosed with cancer and almost 10 million cancer deaths occurred. Further, 28.4 million people are expected to be diagnosed with cancer in 2040, a 47% increase from 2020; as populations across the world age, cancer rates are expected to increase (Sung *et al.*, CA Cancer J Clin 71(3): 209-249, 2021). There is thus an urgent need for new and improved therapies for cancer.

As is becoming increasingly recognised, different cancers can be associated with different mechanistic aberrations which occur in cells, which open up different therapeutic approaches for different groups of cancers. One such group of cancers is those associated with aberrant Wnt/ $\beta$ -catenin signalling.

$\beta$ -catenin is a protein which plays a structural role in cell-cell adhesion. It is also a transcription factor in a Wnt signalling pathway. The Wnt/ $\beta$ -catenin pathway, also known as the canonical Wnt pathway, usually highly conserved, is activated via the binding of extracellular Wnt ligands (including Wnt3a, Wnt1 and Wnt5a) to target membrane receptors via autocrine or paracrine mechanisms. Following activation, the Wnt pathway stabilises  $\beta$ -catenin and transfers it to the nucleus, subsequently facilitating the expression of genes involved in key cellular mechanisms including cell proliferation, differentiation, migration, survival, renewal, and apoptosis (Liu *et al.*, Sig Transduct Target Ther 7(3), 2022).

In the context of cancer, mutations have been documented in various genes of the pathway, and abnormal Wnt/ $\beta$ -catenin signalling has been reported to be involved in the pathogenesis of cancer by facilitating tumorigenesis, cancer cell renewal, proliferation and immune responses. Specifically,  $\beta$ -catenin activation is believed to drive immune cell

exclusion, facilitate cold tumour formation in the tumour microenvironment and is considered to be a key factor of immune-checkpoint inhibition resistance (Pai *et al.*, J Hematol Oncol 10(1): 101, 2017; Tissier *et al.*, Cancer Res 65:(17), 2005).

5 Aberrant Wnt/ $\beta$ -catenin signalling has been associated with cancers of a number of different types, i.e. in a number of different tissues. These include specific forms of colorectal cancer (CRC), melanoma, desmoid tumours, lung cancer, kidney cancer, liver cancer, breast cancer, ovarian cancer, and adrenocortical carcinoma (ACC) (Pai *et al.*, 2017 (supra); Tissier *et al.*, 2005 (supra); and Koury *et al.*, Stem Cells Int:2925869, 2017).

10 ACC is a cancer of the outer layer of the adrenal glands. It is a rare cancer, with an incidence of 0.7-2 per million population/year, and patients diagnosed with ACC have a very poor prognosis; upon progressing on standard of care the median survival is less than a year, and progression free survival is < 2 months in those with advanced or metastatic disease. The treatment of ACC is challenging due to the rarity and malignancy of the disease, and surgical resection is the only curative option for patients with early stage ACC.

15 Patients with ACC also have a high risk of recurrence, whether local or distant, after a complete resection. Unfortunately, ACC is only modestly responsive to standard cytotoxic chemotherapies such as radiotherapy and ablation, and the adjuvant treatment approved for ACC, mitotane, has a low therapeutic index (see e.g. Tissier *et al.*, 2005, supra). ACC thus poses a particular unmet need for cancer therapy. However, more generally, there is an on-

20 going need for new therapies for  $\beta$ -catenin-driven cancers; despite the contribution of Wnt signalling to tumourigenesis having been recognised, and Wnt signalling inhibition having shown promising effects in some preclinical models, no Wnt signalling-targeting drugs have proved clinically successful in cancer or other diseases.

$\beta$ -catenin has been an elusive drug target thus far. Despite their initial promise, the

25 results of trials of therapeutics designed to target an aberrant activated Wnt/ $\beta$ -catenin signalling pathway for the treatment of cancer have been mixed (Zhang & Wang, J Hematol Oncol 13(1): 165, 2020). Various inhibitors targeting the Wnt/ $\beta$ -catenin pathway are currently in development in both preclinical and clinical trials. Diverse categories of targeted agents exist and include Wnt antagonists, porcupine (PORCN) inhibitors,  $\beta$ -catenin/T cell-specific (TCF) inhibitors, and monoclonal antibodies against the receptor protein Frizzled (FZD).

30 Though some candidates appear promising, safety, efficacy and drug delivery concerns remain, and successes have typically only been seen in a relatively small proportion of patients or in patients with only very specific types of cancers. Even where some efficacy is shown, adverse effects are, at present, common, and it is unclear how the activity of some

35 drug candidates can translate into sustained clinical outcomes across multiple cancers associated with an activated aberrant Wnt/ $\beta$ -catenin pathway.

Thus, in view of the widespread involvement of the Wnt/ $\beta$ -catenin pathway in many and diverse cellular processes in different cell types, and its interaction with other signalling pathways, the Wnt/ $\beta$ -catenin pathway presents a challenging target for therapy, in terms of achieving a desired therapeutic benefit, whilst avoiding undesirable side-effects.

5 WO 2011/092347 discloses oligopeptidic compounds which are selectively cytotoxic for neoplastic cells and proposes their use to treat a range of cancers. These oligopeptidic compounds include peptides consisting of the amino acid sequence set forth in SEQ ID NO: 1 (named CyPep-1). The peptides are cationic and show a high ability to bind to negatively-charged membranes, such as are typical of many neoplastic cells, including  
10 particularly cancer cells, resulting in strong and selective cytolytic activity against neoplastic/cancer cells (non-neoplastic/non-cancerous mammalian cells tend to have membranes with a more neutral charge, and are not targeted by CyPep-1). The peptide has not only been shown to be selectively cytotoxic against a variety of cancer cell lines *in vitro* leading to neoantigen release, it has also been shown to have a strong anti-tumour effect  
15 against various types of cancer, and indeed also against non-malignant neoplastic lesions such as warts, and to be well tolerated in animal models of disease. The cytolytic activity of CyPep-1 is also manifest against bacterial cells (possibly also due to the negative charge held by many bacterial cell membranes), and it has been shown to have a potent bactericidal effect against medically-relevant species of Gram-positive and Gram-negative bacteria. Its  
20 use as an antimicrobial agent, including anti-bacterial and anti-fungal, has been proposed (see WO 2011/092347).

#### Summary of the invention

In the course of investigating the onco-therapeutic potential of CyPep-1, the present  
25 inventor has surprisingly found that CyPep-1 can elicit a beneficial therapeutic effect and be used to treat a particular group (i.e. type or class) of cancers, namely those associated with aberrant activation of the Wnt/ $\beta$ -catenin pathway, and in particular ACC.

As shown in the Examples below, CyPep-1 was astonishingly effective in treating patients with ACC, with a far-greater than expected response. This led the inventor to  
30 investigate and consider further the properties of CyPep-1, which suggest that it is functioning as an inhibitor of the Wnt/ $\beta$ -catenin pathway, to reverse immune responses in the tumour microenvironment, i.e. to initiate 'immune reprogramming'. Specifically, as shown in the Examples, CyPep-1 is able to decrease nuclear accumulation of  $\beta$ -catenin and downregulate Wnt-induced Secreted Protein-1 (WISP-1/CCN). This gene is transcribed by  
35  $\beta$ -catenin, and the downregulation is caused by inhibition of the  $\beta$ -catenin pathway by CyPep-1. Thus, the present inventor has discovered a further mode of action for CyPep-1.

Further, nuclear localization of  $\beta$ -catenin is decreased after treatment with CyPep-1, and evidence of activation of Axin2 by CyPep-1 has also been seen. The results in ACC, and other cancers, provide proof of concept for its activity and validation of the CyPep-1 platform with its dual mechanism of action as a new treatment option for many cancer patients with a previously difficult to treat group of cancers.

The present developments thus provide a new and highly advantageous therapy using the CyPep-1 peptide to treat a particular type, or sub-group, of cancer, namely cancers associated with the Wnt/ $\beta$ -catenin pathway.

Accordingly, in a first aspect provided herein is an oligopeptidic compound comprising a D-amino acid sequence as set forth in SEQ ID NO: 1, or a D-amino acid sequence having at least 85 % sequence identity thereto, for use in the treatment of cancer in a subject, wherein said cancer is associated with aberrant activation of the Wnt/ $\beta$ -catenin pathway.

The oligopeptidic compound which is provided for the medical uses and methods herein is accordingly a compound comprising an inverso amino acid sequence.

In an embodiment, every amino acid of the oligopeptidic compound is a D-amino acid. In this embodiment, the oligopeptidic compound is accordingly an inverso oligopeptidic compound.

In an embodiment, the oligopeptidic compound is a D-amino acid peptide having, or consisting of, the sequence as shown in SEQ ID NO: 1.

The oligopeptidic compound may advantageously be used in combination therapy together with one or more other onco-therapeutic agents.

Hence, in another aspect, provided herein is an oligopeptidic compound comprising a D-amino acid sequence as set forth in SEQ ID NO: 1, or a D-amino acid sequence having at least 85 % sequence identity thereto, for the treatment of cancer associated with aberrant activation of the Wnt/ $\beta$ -catenin pathway, optionally wherein every amino acid of the compound is a D-amino acid, and wherein said compound is used in combination with a second therapeutic agent effective to treat said cancer.

A related aspect provides a pharmaceutical product comprising (i) an oligopeptidic compound comprising a D-amino acid sequence as set forth in SEQ ID NO: 1, or a D-amino acid sequence having at least 85 % sequence identity thereto, optionally wherein every amino acid of the compound is a D-amino acid; and (ii) a second oncotherapeutic agent as a combined preparation for separate, simultaneous or sequential use in the treatment of cancer in a subject, wherein said cancer is associated with aberrant activation of the Wnt/ $\beta$ -catenin pathway.

A further related aspect provides a method of treating a cancer associated with aberrant activation of the Wnt/ $\beta$ -catenin pathway comprising administering to a subject in

need thereof an oligopeptidic compound comprising a D-amino acid sequence as set forth in SEQ ID NO: 1, or a D-amino acid sequence having at least 85 % sequence identity thereto, optionally wherein every amino acid of the compound is a D-amino acid, optionally together with a second therapeutic agent.

5 In particular, the oligopeptidic compound, and optionally (i.e. where used) the second therapeutic agent, are administered to the subject in an effective amount. More particularly, in the case of combination therapy, the effective amounts are effective to treat the cancer when administered in combination.

In another aspect, provided herein is use of an oligopeptidic compound comprising a  
10 D-amino acid sequence as set forth in SEQ ID NO: 1, or a D-amino acid sequence having at least 85 % sequence identity thereto, in the manufacture of a medicament for treating cancer associated with aberrant activation of the Wnt/ $\beta$ -catenin pathway, optionally in combination with a second therapeutic agent, and optionally wherein every amino acid of the compound is a D-amino acid.

15 Thus, more particularly this aspect can be seen to provide use of an oligopeptidic compound comprising a D-amino acid sequence as set forth in SEQ ID NO: 1, or a D-amino acid sequence having at least 85 % sequence identity thereto, in the manufacture of a medicament for treating cancer associated with aberrant activation of the Wnt/ $\beta$ -catenin pathway, optionally wherein every amino acid of the compound is a D-amino acid, and  
20 wherein the oligopeptidic compound is used in combination with a second therapeutic agent.

This aspect can also, in other words, be seen to provide use of (i) an oligopeptidic compound comprising a D-amino acid sequence as set forth in SEQ ID NO: 1, or a D-amino acid sequence having at least 85 % sequence identity thereto, optionally wherein every amino acid of the compound is a D-amino acid, and (ii) a second therapeutic agent, in the  
25 manufacture of a medicament for treating cancer associated with aberrant activation of the Wnt/ $\beta$ -catenin pathway.

In this context, the medicament can be seen as a kit, or a pharmaceutical product, comprising first and second therapeutic agents defined as (i) and (ii) above, provided for said use. More broadly, in the medical uses above, where the oligopeptidic agent is used in a  
30 combination therapy with another therapeutic agent it can be viewed as the first therapeutic agent.

In the combination therapies herein, the second therapeutic agent can be any oncotherapeutic agent, in particular any second therapeutic agent effective to treat the cancer. In an embodiment, the second therapeutic agent is an immunotherapeutic agent. IN  
35 a more particular embodiment, it is a checkpoint inhibitor.

In an embodiment, the cancer is ACC.

In another aspect, the invention provides a method of identifying a subject for treatment of a cancer with an oligopeptidic compound comprising a D-amino acid sequence as set forth in SEQ ID NO: 1, or a D-amino acid sequence having at least 85 % sequence identity thereto, optionally wherein every amino acid of the compound is a D-amino acid, said method comprising determining, in a sample obtained from a subject, the presence of a biomarker indicative of an activated Wnt/ $\beta$ -catenin pathway.

In a particular embodiment, the invention provides a method of identifying and treating cancer associated with aberrant activation of the Wnt/ $\beta$ -catenin pathway in a subject, said method comprising:

(i) identifying a subject for treatment of the cancer by determining, in a sample obtained from the subject, the presence of a biomarker indicative of an activated Wnt/ $\beta$ -catenin pathway; and

(ii) administering to said subject an oligopeptidic compound comprising a D-amino acid sequence as set forth in SEQ ID NO: 1, or a D-amino acid sequence having at least 85 % sequence identity thereto, optionally wherein every amino acid of the compound is a D-amino acid, optionally together with a second therapeutic agent.

Thus, a subject with cancer is tested to determine whether or not the cancer is associated with aberrant activation of the Wnt/ $\beta$ -catenin pathway by determining whether or not a biomarker indicative of an activated Wnt/ $\beta$ -catenin pathway is present, and if it is, the oligopeptidic compound is administered to treat the cancer.

The biomarker may be a mutation in a gene of the pathway, and/or a change (e.g. an increase or decrease) in the expression of the gene, and/or a change (e.g. an increase or decrease) in the expression of a protein encoded by the gene (i.e. an increase or decrease in a level of the gene product).

This may include any gene in the pathway, including genes encoding proteins such as  $\beta$ -catenin or Axin2, or in genes which encoding regulators in the pathway. In one representative embodiment, the gene may encode a protein involved in the Axin2 regulatory network. For example, the biomarker may be one or more mutations in a gene encoding Axin2 or a regulator of Axin2. which contains one or more mutations. However, the biomarker is not restricted to Axin2/Axin2 regulatory genes, and may be any gene in the pathway which can be shown to be associated with a clinical response, or disease control.

#### Detailed Description

The present invention and disclosure concern a new use for the previously-identified oligopeptidic compounds of WO 2011/092347, and specifically the peptide CyPep-1 (SEQ ID NO: 1), identified as onco-therapeutic agents based on their selective cytolytic activity

against cancer cells. We have now found that CyPep-1 has additional activity to induce tumour immune-specific activation and reverse immune exclusion. The compounds are proposed herein to treat a new group of patients, namely those with a cancer associated with an aberrant Wnt/ $\beta$ -catenin pathway.

- 5           SEQ ID NO: 1 is a 27-amino acid peptide which consists of a fragment of the tumour suppressor protein Conductin/Axin2, specifically the RGS domain, aa 126-140, coupled to the C-terminus of a HIV-TAT cell-penetrating peptide:

YGRKKRRQRRRGKTLRVAKAIYKRYIE (SEQ ID NO: 1)

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The aforementioned fragment of Conductin/Axin2 has the amino acid sequence KTLRVAKAIYKRYIE (SEQ ID NO: 2; corresponding to amino acid numbers 13-27 of SEQ ID NO: 1) and the HIV-TAT cell-penetrating peptide has the amino acid sequence YGRKKRRQRRRG (SEQ ID NO: 3; corresponding to amino acid numbers 1-12 of SEQ ID  
15 NO: 1).

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Conductin/Axin2 is a central protein in the Wnt/ $\beta$ -catenin pathway in tumourigenesis. It is a member of the  $\beta$ -catenin destruction complex, that targets  $\beta$ -catenin for proteasomal degradation. Axin2 is thus an inhibitor of  $\beta$ -catenin - it inhibits  $\beta$ -catenin activity and induces its degradation. The Axin protein family can also directly facilitate export of  $\beta$ -catenin from  
20 the nucleus.

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The Wnt/ $\beta$ -catenin pathway is highly complex and regulated by several components (i.e. proteins, genes, etc.), and, as discussed previously, Axin2 is a target gene of  $\beta$ -catenin. High levels of expression of  $\beta$ -catenin result in high levels of expression of Axin2, and this pattern of expression is what is observed in  $\beta$ -catenin driven cancers, such as colorectal  
25 cancer. Axin2 is a negative regulator of the Wnt/ $\beta$ -catenin pathway, as Axin2 promotes the phosphorylation and degradation of  $\beta$ -catenin, resulting in a decrease in the amount/levels of  $\beta$ -catenin in the cell. Cancerous cells may 'escape' this negative feedback, i.e. overcome the degradation/inhibition/decrease of  $\beta$ -catenin due to high levels of expression of Axin2, by mutations which inactivate either Axin2 or genes which stabilise Axin2 (e.g. GNAI2/3), a  
30 mechanism known as mutational inactivation. This mechanism results in cancer cells exhibiting high levels of expression of inactivated Axin2, in which the RGS domains of Axin2 are aggregated. As such, large amounts, or 'reservoirs', of Axin2 are present in cancer cells. The compounds herein, exploit, or take advantage of this feature of  $\beta$ -catenin driven cancers.

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35           Thus, the peptide of SEQ ID NO: 1, CyPep-1, has two domains, a first domain, the Conductin/Axin2 domain, derived from a protein involved in the Wnt/ $\beta$ -catenin pathway, and a second domain containing a cationic plasma membrane binding domain that specifically

engages with negatively charged phospholipids like phosphatidylserine (PS). The polar heads of PS are normally only found on the inside of cells, but are found on the outside of cancer cells. As such, PS represents a cancer-specific target. Binding to PS by CyPep-1 leads to aggregation and pore formation through the membrane, leading to the release of cancer antigens and lytic cell death. Further, CyPep-1 is believed to activate Axin2 by stabilizing the RGS domain, leading to polymerization and target engagement. In this regard, the RGS domains of Axin2 can aggregate, and in this aggregated form Axin2 is inactive. By binding to the RGS domains, CyPep-1 can reverse this aggregation, and activate Axin2, or promote its active, non-aggregated form. More particularly, in non-aggregated form Axin2 is able to polymerise, or condense, and it is in this polymerised, or condensed, form that Axin2 is active. Data presented in the Examples below demonstrates that CyPep-1 is able to promote the polymerisation of Axin2 into “condensates” or punctates, which are visible microscopically. Activation of Axin2 by CyPep-1 prevents nuclear translocation and activation of  $\beta$ -catenin.

To avoid proteolytic degradation of CyPep-1, we substituted the naturally occurring L-amino acids with their D-amino acid enantiomers, as these are not recognized by serum proteases. This resulted in a stable peptide of 27 D-amino acids with a theoretical isoelectric point of 11.81 and a molecular weight of 3492.16 U (*Genes & Cancer, Vol. 5 (5-6), May 2014*).

Accordingly, the amino acid sequence of SEQ ID NO: 1, or the sequence with at least 85% sequence identity therewith, which is comprised in the oligopeptidic compound proposed for use herein, is a D-amino acid sequence. In other words, the amino acid sequence is composed entirely of D-amino acids (D-aa). It may also be referred to as an inverso amino acid sequence. In an embodiment, the oligopeptidic compound as a whole is composed entirely of D-amino acids, or, in other words, it is a “D-oligopeptidic compound”. It may also be referred to as an inverso oligopeptidic compound. A peptide consisting wholly of L-amino acids is known in the art as an L-peptide, while a peptide consisting wholly of D-amino acids is known in the art as a D-peptide. The term “inverso-peptide” is used to refer to a peptide with the same amino acid sequence as an L-peptide, but consisting wholly of D-amino acids (i.e. a D-peptide with the same sequence as a corresponding L-peptide). An inverso-peptide/oligopeptidic compound has a mirrored structure to its corresponding L-peptide/oligopeptidic compound (e.g. an L-peptide of the same amino acid sequence). Inverso-peptides/compounds can be advantageous for use in a clinical setting (relative to L-peptides/compounds) because they are not generally susceptible to degradation by serum proteases (due to their unnatural conformation inverso-peptides may not be recognised by protease enzymes). The oligopeptidic compound may in particular comprise or consist of a D-peptide consisting of the amino acid sequence set forth in SEQ ID NO: 1.

In this regard, whilst part of the peptide is derived from Axin2, given that it has an inverso (D-aa) structure, it cannot be assumed that the peptide would retain any functional effect, particularly any signalling effect, associated with the biological activity of the parent protein from which it is derived (i.e. any functional/signalling effect of Axin2). It was thus  
5 surprising, and not expected, that the oligopeptidic compound would be able to affect the expression of proteins involved in signalling, e.g. of WISP-1, as shown in the Examples below, and to be able to downregulate (i.e. inhibit) Wnt/ $\beta$ -catenin signalling.

As used herein, the term "oligopeptidic compound" means a compound which is composed of amino acids or equivalent subunits, which are linked together by peptide or  
10 equivalent bonds. Thus, the term "oligopeptidic compound" includes peptides and peptidomimetics.

By "equivalent subunit", it is meant a subunit which is structurally and functionally similar to an amino acid. The backbone moiety of the subunit may differ from a standard amino acid, e.g. it may incorporate one or more nitrogen atoms instead of one or more  
15 carbon atoms.

By "peptidomimetic", it is meant a compound which is functionally equivalent or similar to a peptide and which can adopt a three-dimensional structure similar to its peptide counterparts, but which is not solely composed of amino acids linked by peptide bonds. A preferred class of peptidomimetics are peptoids, i.e. *N*-substituted glycines. Peptoids are  
20 closely related to their natural peptide counterparts, but they differ chemically in that their side chains are appended to nitrogen atoms along the molecule's backbone, rather than to the  $\alpha$ -carbons as they are in amino acids.

Peptidomimetics typically have a longer half-life within a patient's body, so they are preferred in embodiments where a longer lasting effect is desired. This can help reduce the  
25 frequency at which the composition has to be re-administered. However, for bio-safety reasons a shorter half-life may be preferred in other embodiments; in those embodiments peptides are preferred.

Preferably, the oligopeptidic compound is an oligopeptide (more specifically, a D-oligopeptide). The oligopeptidic compound may incorporate di-amino acids and/or  $\beta$ -amino  
30 acids. Most preferably, the oligopeptidic compound consists of  $\alpha$ -amino acids.

An oligopeptide is a polymer formed from amino acids joined to one another by peptide bonds. As defined herein, an oligopeptide comprises at least three amino acids, though clearly an oligopeptidic compound for use herein comprises more than three amino acids. An oligopeptidic compound or oligopeptide as defined herein has no particular  
35 maximum length, e.g. it may comprise up to 30, 40, 50 or 100 amino acids or more, but typically the prefix "oligo" is used to designate a relatively small number of subunits such as amino acids, i.e. less than 200, preferably less than 100, 90, 80, 70, 60 or 50 subunits. The

oligopeptidic compound of the invention may thus comprise at least 23 and no more than 200 subunits. In embodiments it comprises at least 24, 25, 26 or 27 subunits. Alternatively defined it comprises no more than 50, 45, 40, 35, 30, 29, 28 or 27 subunits. The

oligopeptidic compound may thus comprise a number of subunits in a range composed of  
5 any of the integers set out above for a minimum or maximum number of sub-units.  
Representative subunit ranges thus include 23-150, 23-100, 23-80, 23-50, 23-40, 23-30, 25-  
150, 25-100, 25-80, 25-50, 25-40, 25-30, 26-150, 26-100, 26-80, 26-50, 26-40, 26-30, 27-  
150, 27-100, 27-80, 27-50, 27-40, 27-30, 27-29 and 27-28.

An oligopeptidic compound as defined herein may be simply an oligopeptide, i.e. a  
10 polymer consisting of amino acids joined by peptide bonds. Alternatively, the oligopeptidic  
compound may comprise additional functional groups, conjugates, etc.

The oligopeptidic compound for use herein comprises the amino acid sequence set  
forth in SEQ ID NO: 1, or an amino acid sequence having at least 85 %, 90 % or 95 %  
sequence identity thereto. Such a sequence with at least 85% sequence identity may for  
15 convenience be referred to as a substantially identical, or an equivalent, sequence. In a  
particular embodiment, the oligopeptidic compound comprises the amino acid sequence set  
forth in SEQ ID NO: 1. In another embodiment, the oligopeptidic compound consists of the  
amino acid sequence set forth in SEQ ID NO: 1, or an amino acid sequence having at least  
85 %, 90 % or 95 % sequence identity thereto. In another embodiment, the oligopeptidic  
20 compound consists of the amino acid sequence set forth in SEQ ID NO: 1.

The level of sequence identity between two sequences (e.g. an oligopeptide  
sequence and the sequence set forth in SEQ ID NO: 1) may be determined by performing a  
sequence alignment. A sequence alignment may be performed using any suitable method,  
for instance a computer programme such as EMBOSS Needle or EMBOSS stretcher (both  
25 Rice, P. *et al.*, *Trends Genet.* 16(6): 276-277, 2000) may be used for pairwise sequence  
alignments while Clustal Omega (Sievers, F. *et al.*, *Mol. Syst. Biol.* 7:539, 2011) or MUSCLE  
(Edgar, R.C., *Nucleic Acids Res.* 32(5):1792-1797, 2004) may be used for multiple sequence  
alignments. Such computer programmes may be used with the standard input parameters,  
e.g. the standard Clustal Omega parameters: matrix Gonnet, gap opening penalty 6, gap  
30 extension penalty 1; or the standard EMBOSS Needle parameters: matrix BLOSUM62, gap  
opening penalty 10, gap extension penalty 0.5. Any other suitable parameters may  
alternatively be used.

In addition to the D-amino acids of SEQ ID NO: 1 (or equivalent sequence), the  
oligopeptidic compound may comprise one or more other amino acids, including L-amino  
35 acids, or human-engineered amino acids or natural non-proteinogenic amino acids, e.g.  
amino acids formed through metabolic processes. Examples of non-proteinogenic amino  
acids which may be used include ornithine (a product of the urea cycle) and artificially-

modified amino acids such as 9H-fluoren-9-ylmethoxycarbonyl (Fmoc)-, *tert*-Butyloxycarbonyl (Boc)-, and 2,2,5,7,8-pentamethylchromane-6-sulphonyl (Pmc)-protected amino acids, and amino acids having the carboxybenzyl (Z) group.

*In vitro* and/or *in vivo* stability of the oligopeptidic compounds may be improved or enhanced through the use of stabilising or protecting means known in the art, for example the addition of protecting or stabilising groups, incorporation of amino acid derivatives or analogues or chemical modification of amino acids. Such protecting or stabilising groups may for example be added at the N and/or C-terminus. An example of such a group is an acetyl group and other protecting groups or groups which might stabilise a peptide are known in the art.

As reported in WO 2011/092347, oligopeptidic compounds as defined herein have activity in inhibiting the growth and/or viability of cancer cells. "Inhibiting the growth" of a cell means that any aspect of the growth of the cell, be that an increase in the size of the cell or in the amount and/or volume of its constituents, but more particularly an increase in the numbers of a cell, is reduced, more particularly measurably reduced. The term "growth" thus explicitly includes replication or reproduction of a cell. The rate of growth of a cell, e.g. in terms of the rate in increase of cell number, may be reduced. By way of representative example, growth (e.g. cell numbers, or rate of growth) may be reduced by at least 50, 60, 70, 80, 90 or 95 %. In certain cases, growth may be reduced by 100 %, i.e. growth may be completely inhibited and cease. Thus, replication or reproduction of the cell may be reduced or inhibited. As described, the term "inhibit" includes any degree of reduction of growth.

Inhibition of cell growth may be identified by comparing the rate of growth of a control cell or cell population cultured under standard laboratory conditions and in the absence of an oligopeptidic compound of interest with the rate of growth of an identical or corresponding cell or cell population cultured in the presence of an oligopeptidic compound of interest but in otherwise identical conditions to the control cell or cell population. The rate of cellular replication or reproduction may in particular be assessed by determining cell numbers at a chosen time point. A reduction in cell number in the population cultured in the presence of the oligopeptidic compound relative to the number of cells in the control population indicates that the oligopeptidic compound has activity in inhibiting cell growth. Cell number (and thus growth or otherwise) may be determined by cell counting, e.g. using a haemocytometer.

"Inhibiting the viability" of a cell includes any effect which reduces the viability of a cell, or which renders it less likely to survive or non-viable. The viability of a cell may be viewed as the ability of a cell to survive under given conditions. Inhibition of viability of a cell in particular includes killing or destroying the cell, i.e. causing it to die. Cell death may be assessed by any standard laboratory technique. For instance, failure of a cell or cell population to grow, including to replicate, or to utilise or assimilate nutrients, may be

considered indicative of cell death (i.e. lack of viability). Cell viability may also be assessed by monitoring morphological changes to the cell, or to tissue in which the cell is contained e.g. a tumour. Morphological changes may be analysed by microscopy, for example necrosis or cell lysis may be evident upon visual analysis of cells or tissue, indicating a lack of viability. Typically, a cell can be considered dead if cell membrane integrity is lost.

Inhibition of viability may for instance be identified by comparing the viability of a control cell or cell population incubated under standard laboratory conditions and in the absence of an oligopeptidic compound of interest with the viability of an identical or corresponding cell or cell population incubated in the presence of an oligopeptidic compound of interest but otherwise under identical conditions to the control cell or cell population. Cell viability is commonly assessed using a crystal violet assay, as known to the skilled person. In such an assay, a cellular monolayer adherent to a surface (e.g. a culture plate) is contacted (or not) with a compound of interest. Cell death leads to detachment of cells from the surface. Following contacting with the compound of interest, the monolayer is washed to remove detached cells and then stained with crystal violet, which binds proteins and DNA and thus stains cells. The level of staining can be used to determine viability, i.e. if a cell population contacted with a compound of interest is stained less than a control population, the compound of interest can be considered to inhibit the viability of cells. The level of crystal violet staining of a cell population may be determined visually (simply by eye) or quantitatively by dye extraction using methanol, followed by determination by spectroscopy of the optical density of the methanol-extracted dye at 570 nm.

Many other methods for determining the viability or growth of cancer cells are well known in the art, and many routine assays are available to determine if a cell is alive (viable) or dead. One option is to visually assess cells of interest for morphologies characteristic of cell death, e.g. necrotic or apoptotic bodies, membrane blebs, nuclear condensation and cleavage of DNA into regularly sized fragments, rupturing of cell membranes and leakage of cell contents into the extracellular environment. Other methods exploit the characteristic loss of cell membrane integrity in dead cells. Membrane-impermeable dyes (e.g. trypan blue and propidium iodide) are routinely used to assess membrane integrity. These dyes are excluded from intact cells and so no staining occurs in such cells. If cell membrane integrity is compromised, these dyes can access the cells and stain intracellular components. Alternatively, or in addition, dyes that only stain cells with intact membranes may be used to give an indication of the viability of a cell. The LIVE/DEAD cell viability assay available from Thermo Fisher Scientific is an assay that uses two dyes of different colours, one to stain dead cells, the other to stain live cells, thus enabling each to be identified. Examples of suitable live cell-specific dyes include calcein AM (green) and C12-resazurin (red); examples of suitable dead cell-specific dyes include ethidium homodimer-1 (red), propidium iodide

(red) and SYTOX Green. Another approach to assessing membrane integrity is to detect the release of cellular components into the culture media, e.g. lactate dehydrogenase.

A still further option is to measure the metabolism of the cell. This can be done routinely in a number of ways, for instance the levels of ATP can be measured. Only living  
5 cells with intact membranes can synthesise ATP and because ATP is not stored in cells, levels of ATP drop rapidly upon cell death. Monitoring ATP levels therefore gives an indication of the status of the cell. A yet further option is to measure the reducing potential of the cell. Viable cells metabolising nutrients produce reducing agents (e.g. NADH and NADPH) and accordingly by applying a marker that gives different outputs whether in  
10 reduced or oxidised form (e.g. a fluorescent dye) to the cell, the reducing potential of the cell can be assessed. Cells that lack the ability to reduce the marker can be considered to be dead. The MTT and MTS assays are convenient examples of this type of assay.

A cancer cell divides in an unchecked manner, and may be “immortal”, that is to say telomerase-expressing and hence able to continue dividing *ad infinitum*, rather than dying or  
15 becoming senescent as does a healthy cell after reaching its Hayflick limit. The skilled person is able to determine whether a particular cell is cancerous or healthy. Cancer cells often display distinguishing histological features enabling their identification, e.g. large and irregular nuclei and abnormalities within the cytoplasm. Determination of whether a cell is cancerous may also be performed by genetic testing.

20 The oligopeptidic compound for use herein has activity in inhibiting the growth and/or viability of both *in vivo* and *in vitro* cancer cells. Determination of this activity may conveniently be performed *in vitro* using a suitable cell line. Many laboratory cell lines are cancerous, which due to their “immortality” are convenient for research uses. Any such cancer cell line may be used to determine the activity of a compound of interest, e.g. the cell  
25 lines A172 (human glioblastoma), GAMG (human glioblastoma), U87 (human glioblastoma), 4T1 (murine mammary carcinoma), HOS (human osteosarcoma) and MC38 (murine colon carcinoma). Many others are also known to the skilled person. Such cells may be obtained from any suitable source, e.g. a cell depository such as the ATCC (USA). The activity of a compound of interest is preferably determined using mammalian cancer cells. Human  
30 cancer cells may be used. In particular, the cell line may be derived from a cancer which is associated with aberrant activation of the Wnt/ $\beta$ -catenin pathway. Such cancers are listed below, and a cell line may be derived from any of these.

Cancer cells for testing may also be obtained from a subject, e.g. a human cancer patient. Cancer cells may be surgically removed from a cancer patient and the activity of an  
35 oligopeptidic compound of interest tested thereupon. Thus, the cancer cells may be from a cancer cell line, or derived from a clinical sample or veterinary sample. The cancer cells may

be derived from a tumour. The cancer cells may be from any cancer, but particularly from a cancer that is associated with aberrant activation of the Wnt/ $\beta$ -catenin pathway.

As noted above, the oligopeptidic compound is selectively cytotoxic towards cancer cells. The term "cytotoxic" as used herein has essentially the same meaning as "inhibiting the viability of" as described above. In other words, the oligopeptidic compound selectively inhibits the viability of, or kills, cancer cells (or more preferably inhibits the viability of neoplastic cells generally).

A compound can be said to be selectively cytotoxic towards cancer cells if it has a greater cytotoxic effect against cancer cells than against non-cancerous cells, in particular if it has a greater cytotoxic effect against cancer cells than against healthy cells. Particularly the oligopeptidic compound has no or minimal effect on healthy, non-cancerous cells, but is cytotoxic towards cancer cells.

Methods by which the effect of a compound of interest on cell growth and viability may be analysed are described above. Whether a compound of interest is selectively cytotoxic against cancer cells may be determined by the same method. The viability of a cancer cell population contacted with a compound of interest is compared to the viability of a population of healthy cells contacted with a compound of interest. If, following contacting with a compound of interest under identical conditions, the viability of the cancer cell population has been reduced more than the viability of the population of healthy cells, the compound of interest can be said to be selectively cytotoxic towards cancer cells.

As mentioned above, the present medical uses and methods are predicated on the finding of an additional mode of action of the oligopeptidic compounds, namely their selective activity in inhibiting the Wnt/ $\beta$ -catenin pathway. This activity can be assessed, according to methods known in the art, by assessing the effect of the compounds on signalling through the pathway, for example in *in vitro* tests in cell lines. Downstream effects of inhibiting the pathway may also be assessed. For example, as described in the Example below, the activity of the compound in inhibiting the protein levels and nuclear localization of  $\beta$ -catenin, or downstream target genes like WISP-1, Axin2 and Myc mRNA expression may be determined. Methods for assessing these effects include immunohistochemistry (IHC), Western blots (WB), ELISA, polymerase chain reaction (PCR) and sequencing.

Further, as also described in the Examples below, the effect of the compounds on Axin2 polymerisation may be detected microscopically in *in vitro* cell studies, e.g. using cancer cell lines, using antibodies to visualise Axin2.

An oligopeptidic compound as described herein may be synthesised by the skilled person using standard techniques. Chemical synthesis methods are known for oligopeptidic compounds which comprise D-amino acids or other non-proteinogenic amino acids. Liquid-phase protein synthesis or solid-phase protein synthesis may be used to generate

polypeptides which may form or be comprised within the oligopeptidic compounds for use in the invention. Such methods are well-known to the skilled person, who can readily produce oligopeptidic compounds using appropriate methodology common in the art.

The subject to which the oligopeptidic compound is administered is a subject  
5 suffering from a cancer associated with the aberrant activation of the Wnt/ $\beta$ -catenin pathway. The subject is an animal, which may be a human or any non-human animal, but particularly it is a mammal. This may include, laboratory, domestic, livestock, zoo or sports animals. The subject may be a rodent, such as a mouse, rat, rabbit or guinea pig. The subject may be a pet animal, such as a cat or dog, or a farm animal, such as a horse, cow,  
10 sheep, pig or goat. The subject may be a wild animal, e.g. an animal in a zoo or game park. In a particular embodiment the subject is a primate, such as a monkey or an ape. Most particularly the subject is a human. Thus, the therapy disclosed herein may be for veterinary or clinical purposes, but is preferably for clinical purposes, i.e. for the treatment of a human subject with cancer (i.e., a cancer patient having a cancer caused by the aberrant activation  
15 of the Wnt/ $\beta$ -catenin pathway).

The oligopeptidic compound is to be administered to a subject to treat a cancer associated with aberrant activation of the Wnt/ $\beta$ -catenin pathway.

The term "treat" or "treatment" as used herein refers broadly to any effect or step (or intervention) beneficial in the management of a clinical condition. Treatment may include  
20 reducing, alleviating, ameliorating, slowing the development of, or eliminating the condition or one or more symptoms thereof, which is being treated, relative to the condition or symptom prior to the treatment, or in any way improving the clinical status of the subject. A treatment may include any clinical step or intervention which contributes to, or is a part of, a treatment programme or regimen. Thus "treatment" as used herein encompasses curative  
25 treatment (or treatment intended to be curative), and treatment which is merely life-extending or palliative (i.e. designed merely to limit, relieve or improve the symptoms of a condition).

By "aberrant activation of the Wnt/ $\beta$ -catenin pathway" is meant increased, or abnormal, or in other words perturbed, activation of the pathway, as compared with the pathway in a healthy subject, or in a healthy tissue, or in healthy (i.e. non-cancerous) cells.  
30 Thus, signalling through the pathway is increased in cells of the cancer, e.g. in a tumour or in the local microenvironment of the tumour. In particular, aberrant, or increased activation of the pathway may be characterised by increased protein levels of  $\beta$ -catenin, or increased levels of downstream target genes like WISP-1, Axin2 and Myc, as determined by mRNA expression. Methods for assessing these effects include immunohistochemistry (IHC),  
35 Western blots (WB), ELISA, polymerase chain reaction (PCR). Further, genetic mutations in  $\beta$ -catenin, APC, ZNRF3, MIP1 drive aberrant activation of the pathway and can be detected by sequencing.

The perturbation in the pathway may arise from one or mutations which may occur in one more proteins which are involved in the pathway, for example mutations in a gene encoding a protein selected from:  $\beta$ -catenin, Axin1, Axin2, adenomatous polyposis coli (APC), ZNRF3, MEN1 and GNAI2. This may result in perturbed or abnormal protein expression and/or function. For example, expression of certain proteins may be reduced or increased, or a functional protein may be inactivated (e.g. a suppressor protein, or an enzyme). For example, formation of the degradation complex (DC) which is a feature of the pathway may be reduced. Thus, as will be discussed in more detail below, such a cancer may be identified by screening a sample of the cancer, or a sample from a patient suspected of having such a cancer, for one or more biomarkers indicative of an aberrant, or simply an activated, Wnt/ $\beta$ -catenin pathway.

An aberrant Wnt/ $\beta$ -catenin pathway is known to be associated with numerous types of cancer, although it will be understood that not every cancer of a particular type (e.g. in a particular organ and/or particular tissue) will be associated with the aberrant pathway. In the case of certain cancers, the clinician may know from the state of the art that that particular cancer is associated with aberrant activation of the pathway, without needing to test for it. In some cases, this may be inferred. In other cases, the subject may be screened, or tested, to investigate or determine whether the cancer is associated with an aberrant pathway. This is discussed further below.

A cancer associated with aberrant activation of the Wnt/ $\beta$ -catenin pathway may alternatively be referred to as a  $\beta$ -catenin-driven cancer.

Such cancers may include eye cancer, vulvar cancer, endocrine cancer, including for example adrenocortical tumours, particularly ACC, and parathyroid cancer or thyroid cancer, anal cancer, pancreatic cancer, colorectal cancer, gastric cancer, bile duct cancer, liver cancer, particularly hepatocellular carcinoma (HCC), kidney cancer, e.g. renal cell carcinoma, gallbladder cancer, bladder cancer, skin cancer, including melanoma, although the melanoma can occur anywhere in the body, e.g. uveal melanoma, prostate cancer, penile cancer, breast cancer, head and neck cancer, e.g. head and neck squamous cell cancer (HNSCC), parotid cancer, cervical cancer, oesophageal cancer, endometrial cancer, lung cancer, including small cell and non-small cell lung cancer, e.g. lung adenocarcinoma, glioma, medulloblastoma, ovarian cancer, Wilms' tumour, cholangiocarcinoma, neuroendocrine (carcinoid) cancer, HPV positive cancer, squamous cell carcinoma and sarcoma, or a desmoid tumour.

Squamous cell cancers (SCC) may particularly include HNSCC (listed above), and squamous cell cancer of the cervix, lung, oesophagus, vulva, anus etc.

Particular cancers for treatment according to the uses and methods herein include: ACC, anaplastic thyroid cancer, lung adenocarcinoma, parathyroid carcinoma, head and

neck squamous cell carcinoma, melanoma, e.g. uveal melanoma, neuroendocrine (carcinoid) cancer, vulvar squamous cell carcinoma, chondrosarcoma, anal carcinoma, small cell lung cancer, non-small cell lung cancer.

In certain embodiments, the cancer is selected from: ACC, HNSCC, parotid cancer, parathyroid cancer, thyroid cancer, and uveal melanoma. In other embodiments the cancer is selected from: ACC, parotid cancer, thyroid cancer or parathyroid cancer.

In some embodiments, the cancer is not colorectal cancer, or is not colon cancer or rectal cancer. In a particular embodiment the cancer to be treated is ACC.

Thus, the cancer is particularly a cancer associated with solid tumours, i.e. which manifests clinically as solid tumours. However, the use of the oligopeptidic compound is not restricted to such cases, and includes also other cancers, including haemopoietic cancers, for example acute myeloid leukaemia (AML).

The cancer may thus be any cancer associated with an aberrant Wnt/ $\beta$ -catenin pathway. This includes primary and secondary cancers. As will be discussed in more detail below, the oligopeptidic compounds are particularly effective in treating both primary and secondary tumours or metastases in a subject.

As noted above, the oligopeptidic compound may be used in combination with a second therapeutic agent, particularly a second agent effective to treat the cancer. The second therapeutic agent may be a second anti-cancer agent, though in other embodiments may have a different activity, e.g. it may be anti-inflammatory agent, or any other agent useful for the treatment of the patient.

In a particular embodiment, said second therapeutic agent may be selected from a chemotherapeutic agent, immunotherapeutic agent, hormone therapy, radiation therapy or photodynamic therapy.

As referred to herein a chemotherapeutic agent is a drug which is administered and which acts against the cancer, e.g. which is destructive to malignant cells and tissues. A chemotherapeutic drug is generally a small molecule agent. A typical chemotherapeutic agent is cytotoxic or cytostatic and acts to kill or inhibit the growth of cancer. Any chemotherapy agent of any class may be used, e.g. taxanes (such as paclitaxel and docetaxel), topoisomerase inhibitors (such as topotecan), anthracyclines (such as doxorubicin and epirubicin), nucleoside analogues (such as gemcitabine), platinum-based agents (such as cisplatin and carboplatin), alkylating agents (such as cyclophosphamide) and kinase inhibitors (such as imatinib) or other chemotherapeutic agents or drugs as described hereinbefore.

As referred to herein an immunotherapeutic agent is any agent which affects the immune system, or immune response of the subject. For example, it may be administered to induce, enhance or suppress an immune response. Such immunotherapeutic agents may

include e.g., antibodies, notably monoclonal antibodies, checkpoint inhibitors, cytokines, cells for adoptive cell transfer therapy (ACT), for example natural or modified immune cells, which may be autologous or allogeneic (donor cells), e.g. chimeric antigen receptor (CAR) T-cells, tumour-infiltrating lymphocytes (TILs) obtained or derived from a patient, genetically modified immune cells, NK cells etc., and vaccines.

As referred to herein hormone therapy, also known as hormonal therapy, anti-hormonal therapy, hormone treatment or endocrine therapy, is used to treat cancers that utilise hormones to grow by removing, blocking or adding specific hormones to the body to alter the activity or production of specific hormones. Examples of hormone therapy agents include e.g. aromatase inhibitors, luteinising hormone releasing hormone (LHRH) agonists or blockers or LH blockers, fulvestrant, anti-androgens, gonadotrophin releasing hormone (GnRH) blockers, enzalutamide, abiraterone, darolutamide, medroxyprogesterone acetate and megestrol.

As referred to herein radiation therapy, otherwise known as radiotherapy, refers to the use of high doses of ionising radiation to control or kill cancer cells. Such radiation therapies may include e.g., external beam radiation therapy and internal radiation therapy (such as e.g., brachytherapy etc.), including systemic radiation therapy (such as e.g., radiopharmaceuticals, radioactive iodine etc.)

As referred to herein photodynamic therapy refers to the use of a combination of a light sensitising drug, such as e.g., a photosensitiser (e.g., porfimer sodium) or photosensitising agent, and light which may originate from e.g., a laser or other source, such as a light-emitting diode (LED). Such photodynamic therapies may include e.g., extracorporeal photopheresis (ECP) or photoimmunotherapy (PIT).

In a particular embodiment, said second therapeutic agent is a cytotoxic agent, a cell for adoptive cell transfer therapy, an antibody, a hormone, or a checkpoint inhibitor.

Checkpoint inhibitors are agents which bind to immune checkpoints and inhibit their function. Immune checkpoints are regulators of the immune system which function to promote antigen-specific activation of immune cells and to enable self-tolerance, thus supporting immune activity against antigenic targets and preventing auto-immune disease and aberrant immune system activity against host tissues. Immune checkpoints may be stimulatory or inhibitory. Stimulatory immune checkpoints act to modulate immune cell activity against antigenic targets, by stimulating proliferation and effector responses when bound by their cognate ligand or agonist. Examples of stimulatory immune checkpoints include CD28, which acts as a co-stimulator for T-cell activity and initiates proliferation of T-cells upon binding to its ligands, CD80 and CD86.

Inhibitory immune checkpoints down-regulate or inhibit immune cell function upon binding by their cognate ligand or agonist, promoting self-tolerance and preventing

autoimmune activity or excessive and aberrant immune responses with the potential to cause damage to the host, such as cytokine storms. However, activation of inhibitory immune checkpoints can prevent the immune system from targeting cancer cells. Examples of such inhibitory immune checkpoints include PD-1 and CTLA-4. A checkpoint inhibitor as  
5 defined herein (and generally in the art) is an agent which inhibits the activity of an inhibitory immune checkpoint. With the exception of the paragraph above where its meaning is explicitly defined, throughout the present disclosure the term "immune checkpoint" means an inhibitory immune checkpoint.

As defined herein a checkpoint inhibitor refers to any agent which binds an immune  
10 checkpoint or immune checkpoint ligand and acts directly to prevent activation of the immune checkpoint. Thus, a checkpoint inhibitor may be an antagonist of an immune checkpoint. All currently-available checkpoint inhibitors in clinical use act by blockading their target immune checkpoint, i.e. binding to it or its ligand and thus preventing the interaction between checkpoint and ligand (a mechanism known as immune checkpoint blockade).  
15 However, the checkpoint inhibitor for use herein in combination with the oligopeptidic compound may act by any mechanism, including immune checkpoint blockade, non-competitive inhibition of the immune checkpoint, covalent or structural alteration of the immune checkpoint (or its ligand), etc. Ideally a checkpoint inhibitor should cause cancer cells to be exposed to the immune system without causing that same system to attack  
20 healthy tissue.

A checkpoint inhibitor may thus be any agent which binds to an immune checkpoint or immune checkpoint ligand and inhibits the activity of the immune checkpoint. A checkpoint inhibitor may be for example a small molecule, a ligand antagonist, an affimer or an antibody. An antibody, as referred to herein, may be a natural or synthetic antibody, or a  
25 fragment or derivative thereof. The term "antibody" is used broadly herein to include any type of antibody or antibody-based molecule. This includes not only native antibody molecules but also modified, synthetic or recombinant antibodies, as well as derivatives or fragments thereof. An antibody may thus be any molecule or entity or construct having antibody-based binding region(s), that is a binding domain(s) which is/are derived from an antibody.

Accordingly, an antibody may alternatively be defined as a binding molecule  
30 comprising an antigen-binding domain obtained or derived from an antibody. The antibody may be of, or may be derived from/based on, an antibody of any convenient or desired species, class or sub-type. As noted above, the antibody may be natural, derivatised or synthetic. It may be monoclonal or polyclonal. Thus, the antibody may bind to a single  
35 epitope or it may be a mixture of antibodies (or antibody molecules) binding to different epitopes.

Accordingly, the checkpoint inhibitor may be a binding molecule comprising an antigen-binding domain from an antibody specific for (or directed against) an immune checkpoint or a ligand thereof. Examples of such "antibodies" (i.e. antibody-based binding molecules) include monoclonal and polyclonal antibodies, antibody fragments including Fab, Fab', F(ab')<sub>2</sub> and Fv fragments and any fragment lacking an Fc region, chimeric (e.g. humanised or CDR-grafted) antibodies, single chain antibodies (e.g. scFv antibodies), antibodies identified or obtained from phage display, etc. In a particular embodiment the checkpoint inhibitor is a monoclonal antibody.

An affimer is an engineered non-antibody protein which mimics antibody binding to a target. Affimers are derived from the cystatin protein family, and share a common structure of an  $\alpha$ -helix lying on top of an anti-parallel  $\beta$ -sheet. Affimers, and methods for their generation, are described in WO 2009/136182.

In a particular embodiment the checkpoint inhibitor inhibits the activity of PD-1. The checkpoint inhibitor may in particular block the interaction between PD-1 and PD-L1 (or the interaction between PD-1 and PD-L2), thus preventing PD-1 activation (as described above, PD-1 activation inhibits T-cell effector functionality). A checkpoint inhibitor which blocks the interaction between PD-1 and PD-L1/PD-L2 binds to one of these proteins and prevents interaction between the two proteins from taking place. Thus, a checkpoint inhibitor which blocks the interaction between PD-1 and PD-L1 may bind to PD-1 or may bind to PD-L1 or PD-L2. In particular embodiments, the checkpoint inhibitor binds PD-1 or PD-L1. In particular, such a checkpoint inhibitor may bind to the PD-L1 binding site of PD-1, or the PD-1 binding site of PD-L1. It may be advantageous to use a checkpoint inhibitor which binds PD-1 to block the interaction between PD-1 and its ligands, in order to block interactions between PD-1 and both PD-L1 and PD-L2.

In particular embodiments, the checkpoint inhibitor which blocks the interaction between PD-1 and PD-L1/PD-L2 is an antibody (particularly a monoclonal antibody, or a derivative or fragment thereof) which binds PD-1. In other embodiments, the checkpoint inhibitor which blocks the interaction between PD-1 and PD-L1 is an antibody (particularly a monoclonal antibody, or a derivative or fragment thereof) which binds PD-L1. A number of such antibodies are known in the art, for instance Nivolumab (Bristol-Myers Squibb), a human monoclonal anti-PD1 IgG4 antibody; Pembrolizumab, a humanized IgG4 anti-PD-1 antibody (Merck); Atezolizumab, a fully humanised anti-PD-L1 antibody (Genentech); and Durvalumab, a human anti-PD-L1 antibody (Medimmune/AstraZeneca), have all received regulatory approval and may be used herein. Many other such antibodies are currently in development/trials, such as Tislelizumab, a humanised anti-PD-1 antibody (BeiGene); and Avelumab, a fully human anti-PD-L1 antibody (Pfizer/Merck), and may also be used according to the present invention. Similarly, an antibody (preferably a monoclonal antibody,

or a derivative or fragment thereof) which binds PD-L2 may be used to block the interaction between PD-1 and PD-L2.

As discussed above, another immune checkpoint which may be targeted by a checkpoint inhibitor is CTLA-4. Thus, in another embodiment the checkpoint inhibitor blocks the interaction between CTLA-4 and its ligands CD80 and CD86. As detailed above with respect to the PD-1/PD-L1 interaction, an agent which blocks the interaction between CTLA-4 and CD80/CD86 binds to one of these proteins and prevents CTLA-4 from interacting with CD80 and/or CD86. Such an agent may bind CTLA-4, CD80 or CD86. However, CD80 and CD86 also function as co-stimulatory molecules for T-cells, via binding to CD28. Accordingly, any checkpoint inhibitor which blocks the interaction between CTLA-4 and CD80/CD86 must not block the interaction between CD28 and CD80/CD86. Therefore, a checkpoint inhibitor which blocks the interaction between CTLA-4 and CD80/CD86 preferably binds CTLA-4 rather than CD80 and/or CD86. In particular, a checkpoint inhibitor may bind CTLA-4 at the binding site where it interacts with CD80 or CD86.

In a particular embodiment, the checkpoint inhibitor which blocks the interaction between CTLA-4 and CD80/CD86 is an antibody (preferably a monoclonal antibody, or a derivative or fragment thereof) which binds CTLA-4. A number of such antibodies are known in the art, for instance Ipilimumab, a human IgG1 monoclonal antibody (Bristol-Myers Squibb), which has received regulatory approval. Other such antibodies are in development/trials, for instance Tremelimumab, a human IgG2 monoclonal antibody (Medimmune/AstraZeneca).

PD-1 and CTLA-4 are expressed on T-cells. PD-1 and CTLA-4 inhibition is designed to promote T-cell activity, and so if an antibody targeting PD-1 or CTLA-4 is used as a checkpoint inhibitor, it may be preferable that binding of the antibody to its target does not initiate antibody-dependent cellular cytotoxicity (ADCC), which could cause the death of the target T-cell. ADCC is primarily mediated by natural killer (NK) cells, which express Fc receptors (such as CD16) which recognise and bind the Fc (i.e. constant) domains of antibodies bound to target antigens. Binding of an Fc receptor of an NK cell to the Fc domain of an antigen-bound antibody leads to activation of the NK cell, which releases cytotoxic agents which kill the cell to which the antibody is bound.

Antibodies able to bind target cells without inducing ADCC may be of a particular IgG sub-class which is not associated with ADCC activity, or may be rationally designed by introducing point mutations to inhibit Fc receptor binding. Such rational design is straightforward for the skilled person. For instance, mutation of position 228 in the human IgG4 constant region may prevent Fc receptor binding of the antibody. Thus, Nivolumab and Pembrolizumab (both of which are human IgG4 antibodies, as mentioned above) both contain an S228P mutation in their constant regions which prevents Fc receptor binding,

meaning neither antibody mediates ADCC. Any antibody against PD-1 for use as a checkpoint inhibitor according to the present invention may comprise the same or an equivalent mutation. By equivalent mutation is meant a mutation at a different residue (or a corresponding residue in the constant region of a different antibody isotype) which has the same effect, i.e. inhibition of Fc receptor binding.

However, in other contexts it may be preferred that the checkpoint inhibitor is able to mediate ADCC. The anti-CTLA-4 antibody Ipilimumab has been shown to mediate ADCC against Treg cells, mediated by non-classical CD16-expressing monocytes, thus providing a second mechanism of preventing immune effector cell down-regulation (Romano *et al.*, PNAS 112(19) 6140-6145, 2015).

Though less prominent, other immune checkpoints in addition to PD-1 and CTLA-4 are also known and may be targeted by a checkpoint inhibitor. Such other checkpoints include for instance, LAG-3 (also known as CD223). In particular, such an agent may be an antibody which binds LAG-3, a number of which are in development, such as BMS-986016 (Bristol-Myers Squibb).

In a further alternative approach, an inhibitor of killer cell immunoglobulin-like receptor (KIR) may be used as a checkpoint inhibitor. For example, Lirilumab (Bristol-Myers Squibb) is a fully human monoclonal antibody to KIR which may be used as a checkpoint inhibitor.

Other immune checkpoints which may be targeted by checkpoint inhibitors to prevent their activation, for instance by blocking their interaction with their cognate ligands) include B7-H3 (also known as CD276), BTLA (also known as CD272), VISTA and TIM-3 (also known as HAVCR2). Where appropriate, the ligands of these checkpoints may also be targeted by checkpoint inhibitors in order to block interaction of the ligand with its immune checkpoint receptor. For example, the TIM-3 ligand phosphatidylserine (PS) may be targeted by checkpoint inhibitors to block its interaction with TIM-3, for instance using an anti-PS antibody. An example of such an antibody is Baviximab (Oncologie Inc.), which is currently in development.

Any checkpoint inhibitor may be used. As detailed above, many checkpoint inhibitors are known to the skilled person, or may be developed by e.g. rational design or raising an antibody against an appropriate target. In particular embodiments, more than one checkpoint inhibitor may be used in combination with the oligopeptidic compound. For instance, two or more different checkpoint inhibitors, which each inhibit the activation of different immune checkpoints, may be used. For instance, a checkpoint inhibitor which blocks PD-1 activation may be used in combination with a checkpoint inhibitor which blocks CTLA-4 activation. Use of multiple checkpoint inhibitors in combination has previously been shown to yield

improvement in treatment outcomes in some cancers relative to the use of any single checkpoint inhibitor.

As noted above, the oligopeptidic compound, and indeed any second agent with which it is used in combination, may be administered by any convenient or desired route, which may depend on the subject, the condition, and the nature of the agent etc. The selection of a suitable mode of administration is well within the routine skill of a clinician in this field.

Possible routes of administration include oral, rectal, nasal, topical, vaginal and parenteral administration. Oral administration as used herein includes buccal and sublingual administration. Topical administration as used herein includes transdermal administration. Parenteral administration as defined herein includes subcutaneous, intramuscular, intravenous, intraperitoneal and intradermal administration. The oligopeptidic compound in particular may be administered to the subject for systemic delivery, for example via an oral or parenteral route of administration, or be administered locally to the site of the cancer to be treated, e.g. locally to or directly into a tumour. Possible routes of local administration include topical administration, delivery by direct administration e.g. by injection or infusion to the site of the cancer (e.g. tumour), and inhalation, depending of course on the site of the cancer (tumour).

In a particular embodiment the oligopeptidic compound is administered to the subject by intra-tumoural administration, e.g. by injection or infusion directly into a tumour. This has been found to be particularly advantageous. In the case of the oligopeptidic compounds, intra-tumoural administration into a tumour (e.g. a primary tumour) has been found to cause regression not only of the injected tumour, but also of tumours at other sites (e.g. secondary tumours). Indeed, this has been observed also by injecting a secondary tumour, and seeing regression of tumours elsewhere. This is demonstrated in Figure 9, as shown in the Examples below. Thus, a strong abscopal effect of the oligopeptidic compounds may be seen. Accordingly, the most convenient or accessible, or possibly the largest or most developed etc., tumour may be selected as the administration site. However, other forms of administration are not precluded, including systemic forms of administration.

The commonly accepted principle that metastasis from solid tumours requires systemic administration of anticancer drugs makes anti-intuitive the notion of treating metastatic disease with intra-tumoral administration of drugs. The fact is that systemically administered anticancer drugs are rarely curative for millions of cancer patients treated each year. Liver metastases are associated with poor prognosis as compared with metastasis in other organs and liver metastasectomy and other liver directed procedures such as cryotherapy, thermoablation or chemoembolization are well accepted procedures to prolong the survival of ACC patients. The intra-tumoral administration of drugs has some clear

advantages over systemic administration, including the achievement of several orders of magnitude of intra-tumoral concentration of the drug and potential reduction of the systemic toxicity. The preliminary efficacy data of CyPep-1 in ACC, as reported in the Examples below, suggests that its intra-tumoral administration, including liver metastasis, represents a promising advance in the management of metastatic ACC patients.

Where the oligopeptidic compound is used in combination with another therapeutic agent, the two agents may be administered separately, simultaneously or sequentially. By "separate" administration, as used herein, is meant that the oligopeptidic compound and the second agent are administered to the subject at the same time, or at least substantially at the same time, but by different administrative routes. "Simultaneous" administration, as used herein, means that the oligopeptidic compound and the second agent are administered to the subject at the same time, or at least substantially the same time, by the same administrative route. By "sequential" administration, as used herein, is meant that the oligopeptidic compound and the second agent are administered to the subject at different times. In particular, administration of the first therapeutic agent is completed before administration of the second therapeutic agent commences. When administered to a subject sequentially, the first and second therapeutic agent may be administered by the same administrative route or by different administrative routes.

Administration of the oligopeptidic compound and/or the second agent may be performed repeatedly (i.e. two or more times) during the course of treatment of a subject. For instance, the subject may receive a number of cycles of treatment, in which both the oligopeptidic compound and the second agent are administered. Alternatively, the subject may receive a single dose of one of the therapeutic agents and repeated doses of the other.

If multiple further therapeutic agents are administered to the subject in combination with the oligopeptidic compound, the two or more further agents may be administered separately, simultaneously or sequentially to one another.

In a particular embodiment, the second agent, e.g. a checkpoint inhibitor, is administered parenterally to the subject. For instance, the second agent may be administered to the subject intravenously. In an embodiment, the oligopeptidic compound is administered intra-tumourally, and the second agent, e.g. checkpoint inhibitor, is administered parenterally, e.g. intravenously.

The oligopeptidic compound, and where used second or further therapeutic agent, are formulated for administration according to principles well known in the art. Thus, they are provided the form of a pharmaceutical composition comprising the compound and/or second or further therapeutic agent together with one or more pharmaceutically acceptable carriers or excipients.

As noted above, the oligopeptidic compound and/or the checkpoint inhibitor (or pharmaceutical compositions comprising them) may be administered to the subject in a manner appropriate to the cancer to be treated. The quantity and frequency of administration will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease, although appropriate dosages may be determined by clinical trials. Conveniently the oligopeptidic compound and/or the second agent may be provided to a subject in a daily, weekly or monthly dose, or a dose in an intermediate frequency, e.g. a dose may be provided every 2, 3, 4, 5 or 6 days, every 2, 3, 4, 5 or 6 weeks, every 2, 3, 4, 5 or 6 months, annually or biannually. As noted above, the same dosage regime or different dosage regimes may be used for administration to the subject of the oligopeptidic compound and the second or further agent.

Doses may be administered in amounts dependent on the size of the subject. The oligopeptidic compound may be administered in doses of from 10 µg/kg to 100 mg/kg body mass, e.g. 10 µg/kg to 50 mg/kg body mass, 10 µg/kg to 20 mg/kg body mass, 10 µg/kg to 10 mg/kg body mass, 10 µg/kg to 5 mg/kg body mass, 10 µg/kg to 2.5 mg/kg body mass, 100 µg/kg to 5 mg/kg body mass, 100 µg/kg to 2.5 mg/kg body mass, 500 µg/kg to 5 mg/kg body mass, or 1 mg/kg to 5 mg/kg body mass. In a particular embodiment, the oligopeptidic compound is administered in a dose of about 2 mg/kg body mass, e.g. 1 mg/kg to 2.5 mg/kg body mass, 1.5 mg/kg to 2.5 mg/kg body mass or 1.8 mg/kg to 2.2 mg/kg body mass. The skilled clinician will be able to calculate an appropriate dose for a patient based on all relevant factors, e.g. age, height, weight, the condition to be treated and its severity.

Similar considerations apply to the second or further therapeutic agent. Doses may be administered in amounts dependent on the size of the subject. The second therapeutic agent may be administered in doses of from 10 µg/kg to 100 mg/kg body mass, e.g. 10 µg/kg to 50 mg/kg body mass, 10 µg/kg to 10 mg/kg body mass, 10 µg/kg to 5 mg/kg body mass, 10 µg/kg to 2.5 mg/kg body mass, 100 µg/kg to 5 mg/kg body mass, 100 µg/kg to 2.5 mg/kg body mass, 500 µg/kg to 5 mg/kg body mass, or 1 mg/kg to 5 mg/kg body mass. In a particular embodiment, the second therapeutic agent is administered in a dose of about 2 mg/kg body mass, e.g. 1 mg/kg to 2.5 mg/kg body mass, 1.5 mg/kg to 2.5 mg/kg body mass or 1.8 mg/kg to 2.2 mg/kg body mass. The skilled clinician will be able to calculate an appropriate dose for a patient based on all relevant factors, e.g. age, height, weight, the condition to be treated and its severity.

As noted above, the second agent, e.g. checkpoint inhibitor may be administered at the same dose as the oligopeptidic compound, or may be administered at a higher dose or, in particular, a lower dose to the oligopeptidic compound. The doses may be reduced when the compound and agent are used in combination, over the dose when the compound or agent are used individually (e.g. in monotherapy). For instance, a checkpoint inhibitor may

be administered at a dose of from 100 µg/kg to 100 mg/kg body mass, e.g. 500 µg/kg to 50 mg/kg body mass or 1 mg/kg to 10 mg/kg body mass. Exemplary doses include 1 mg/kg body mass, 2 mg/kg body mass, 3 mg/kg body mass, 4 mg/kg body mass, 5 mg/kg body mass, 6 mg/kg body mass, 7 mg/kg body mass, 8 mg/kg body mass, 9 mg/kg body mass and 10 mg/kg body mass. The checkpoint inhibitor may be administered at a fixed dose, e.g. from 100 mg to 1.5 g. Exemplary doses of checkpoint inhibitor include 100 mg, 200 mg, 240 mg, 250 mg, 300 mg, 400 mg, 480 mg, 500 mg, 600 mg, 700 mg, 800 mg, 900 mg, 1000 mg, 1100 mg, 1200 mg, 1300 mg, 1400 mg and 1500 mg.

Suitable dosage regimes for many checkpoint inhibitors are known. E.g. nivolumab, when used alone, is administered following a dosage regime of 240 mg IV every 2 weeks or 480 mg IV every 4 weeks; ipilimumab, when used alone in melanoma therapy is administered following a dosage regime of 3 mg/kg IV every 3 weeks. These and many other such checkpoint inhibitor dosage regimes are known to the skilled person and may be found within the licensing approvals issued by regulatory bodies such as the FDA and EMA.

For combination therapies the oligopeptidic compound and second or further therapeutic agent may be provided in the form of a kit comprising both, or more, components. For example, the oligopeptidic compound and second therapeutic agent may be provided in separate containers, i.e. in separate compositions, or in a single composition in a single container. Each therapeutic agent may be provided in any appropriate form, e.g. in an aqueous solution or as a lyophilisate.

As noted above, as well as the medical uses herein, also provided are companion diagnostics, that is methods for identifying, or determining, which subjects are suitable or appropriate for treatment by the oligopeptidic compound, according to the medical uses and therapeutic methods herein. It is not essential, or even important, to perform such a screening but it may be desirable or useful in some cases.

Thus, in certain embodiments, it can be determined whether a subject is suffering with a cancer which is associated with aberrant activation of the Wnt/β-catenin pathway. As indicated above, this can be done by determining, in a sample obtained from a subject, the presence of a biomarker indicative of an aberrant or an activated Wnt/β-catenin pathway.

Thus, a subject, e.g. a patient presenting in a hospital with a tumour, or signs of cancer, may as part of their assessment be screened, or tested, to determine whether or not such a biomarker is present. This may be done in on initial presentation, or subsequently during the course of assessment or treatment of a subject, for example, when considering further therapies, or on recurrence etc. or during the course of a therapy.

The sample may be any sample in which such a biomarker is present, and this may depend on the biomarker and/or the cancer in question. For example, the sample may be a sample of cells or tissue from the site of the cancer, for example a tumour biopsy. It may be

a solid or liquid biopsy of the cancer. In other embodiments, it may be sample of body tissue or fluid in which markers of the cancer may be present, for example a blood derived sample, e.g. serum or plasma, which contains circulating cancer DNA, e.g. cell-free DNA.

As noted above, mutations in various genes of the Wnt/ $\beta$ -catenin pathway have been  
 5 identified and reported, and these may have various effects, for example on the expression level of a protein, e.g. a protein may be up- or down-regulated, and/or the function of protein may be altered, e.g. a protein may be inactivated. Thus, the biomarker may be a mutation, or it may be an increase or decrease in the level (i.e. amount) of a particular protein or protein complex, in a particular tissue, or cell, or sub-cellular compartment. For example, the amount  
 10 of  $\beta$ -catenin in the nucleus may be increased. Increased expression may be determined at the level of protein or mRNA. Further the biomarker may be an altered activity of a protein, e.g. an enzyme, and thus functional assays for protein, e.g. enzyme, activity may be performed. Thus, the biomarker may be an increase or decrease in the level of activity of a protein, e.g. enzyme.

15 In an embodiment, said biomarker is:

- (i) an increase in expression of one or more of the following proteins in a tumour sample:  $\beta$ -catenin, WISP-1, FZD3, FZD6, FZD7, Wnt3, Wnt4, Wnt5A, RSPO1, RSPO2, RSPO3, RSPO4, c-MYC, cyclin-D, TCF21;
- (ii) a mutation in one or more genes selected from:  $\beta$ -catenin (CTNNB1), AXIN1,  
 20 AXIN2, APC, ZNRF3, MEN1, GNAI2, RNF43;
- (iii) upregulation of expression of one or more of the following genes: WISP-1, c-MYC, cyclin-D, TCF21, FZD3, FZD6, FZD7, Wnt3, Wnt4, Wnt5A, RSPO2, CLDN1, LGR5.

In another embodiment, said biomarker is selected from any one or more of (i), (ii) and (iii) above, or below:

- 25 (i) an increase in expression of one or more of the following proteins in a tumour sample:  $\beta$ -catenin, WISP-1, Axin2, FZD3, FZD6, FZD7, Wnt3, Wnt4, Wnt5A, RSPO1, RSPO2, RSPO3, RSPO4, c-MYC, cyclin-D, TCF21;
- (ii) a mutation in one or more genes selected from:  $\beta$ -catenin (CTNNB1), AXIN1, AXIN2, APC, APC2, ZNRF3, MEN1, GNAI2, GNAI3, RNF43, DVL1, LPR5, LPR6, MED12,  
 30 and BCL9L;
- (iii) upregulation of expression of one or more of the following genes: WISP-1, Axin2, c-MYC, cyclin-D, TCF21, FZD3, FZD6, FZD7, Wnt3, Wnt4, Wnt5A, RSPO2, CLDN1, LGR5.

In particular representative embodiments, the biomarker may be an increase in the expression of the proteins  $\beta$ -catenin, Wisp1 or Axin2. In another embodiment, the biomarker  
 35 may be an increase in the expression of  $\beta$ -catenin or Wisp1.

Clinical studies may identify which genes/proteins in the pathway may serve as useful biomarkers. Thus, by studying the expression of genes/proteins, or the presence of

mutations in particular genes, or indeed the identify of specific mutations in those genes, in subjects who have responded to treatment with a compound as described herein (e.g. CyPep-1) compared to non-responders, suitable biomarkers may be identified. The identification of suitable biomarker genes in this manner is described in the Examples below.

5 A responder in this respect may be a subject who has exhibited any positive clinical response; this may include complete or partial response, e.g. in terms of tumour regression (such as reduced tumour size and/or number), or in other clinical signs, or in reduced or inhibited disease progression, i.e. stable disease. Thus, for example, suitable biomarkers may be identified in subjects exhibiting partial response or stable disease. This is described  
10 in the Examples below.

By way of representative example, the presence of one or more mutations in one or more of the genes in the following groups of genes may serve as a biomarker according to the methods herein:

- (i) Axin2, DVL1, APC2, LRP5, MEN1, GNAI3, MED12, CTNNB1, LPR6;
- 15 (ii) Axin2, DVL1, APC2, LRP5, GNAI3, MED12, CTNNB1, LPR6;
- (iii) Axin2, DVL1, APC2, LRP5, MEN1, GNAI3, MED12, CTNNB1;
- (iv) Axin2, DVL1, APC2, LRP5, GNAI3, MED12, CTNNB1;
- (v) Axin2, DVL1, APC2, LRP5, MEN1, GNAI3, MED12;
- (vi) Axin2, DVL1, APC2, LRP5, GNAI3, MED12;
- 20 (vii) Axin2, DVL1, APC2, LRP5, MEN1;
- (viii) Axin2, DVL1, APC2, LRP5.

As noted earlier, Conductin/Axin2 is a central protein in the Wnt/ $\beta$ -catenin pathway in tumourigenesis, and CyPep-1 is believed to activate Axin2 to prevent the nuclear translocation and activation of  $\beta$ -catenin. Due to this mechanism of action, patients with one  
25 or more mutations in genes which lead to a decrease in Axin2 activity, or an increase in inactive Axin2 may find particular benefit from treatment with CyPep-1. Accordingly, the presence of one or more mutations in one or more genes encoding a protein involved in the Axin2 regulatory network may represent useful biomarkers for such patients, especially such mutations which inactivate Axin2 or which lead to a decrease in Axin2 activity.

30 Thus, one representative sub-group of biomarkers representative of biomarkers in the Axin2 regulatory network may include the mutations in the genes listed in (v) to (viii) above. Representative mutations in these genes are illustrated in Table 1.

35

Table 1

| Gene             | Mutation   |                |              |                |
|------------------|------------|----------------|--------------|----------------|
|                  | Chromosome | Start position | End position | Base(s) change |
| Axin2            | Chr17      | 65536954       | 65536956     | GCT/G          |
|                  |            | 65541463       | 65541463     | G/T            |
| $\beta$ -catenin | Chr3       | 41224612       | 41224612     | G/A            |
| LRP6             | Chr12      | 12138487       | 12138487     | C/T or G/A     |
| DVL1             | Chr1       | 1336153        | 1336153      | C/T            |
| GNAI3            | Chr1       | 109592203      | 109592203    | A/G            |
| APC2             | Chr19      | 1468062        | 1468062      | C/T            |
| LRP5             | Chr11      | 68416345       | 68416345     | C/T            |
| MED12            | ChrX       | 71130097       | 71130097     | A/C            |

Methods for detecting mutations or for determining expression level of a gene are known widely described in the art, as indeed are methods for determining the amount of a protein of a given protein in a sample etc. Assays for the activity of various proteins involved in the Wnt/ $\beta$ -catenin pathway are described in the art.

#### Description of Figures

The present invention may be more fully understood from the non-limiting Examples below and in reference to the drawings, in which:

- Figure 1 presents results showing that CyPep-1 inhibits the Wnt/ $\beta$ -catenin signaling. A) quantification of nuclear levels of  $\beta$ -catenin in adrenocortical cell line Y1, colorectal cell line CT26 and melanoma cell line B16-F10. B) Quantification of the  $\beta$ -catenin target gene Wnt Induced Secreted protein-1 (WISP-1/CCN4) by PCR in CT26 incubated at different concentrations of CyPep-1 C) Quantification of WISP-1 in the colorectal cell line CT26, melanoma cell lines B16-F10 and Yumm1.7 and glioma cell line GL261. D) Schematics illustrating the mechanism of action (MOA) of CyPep-1 on Wnt/ $\beta$ -catenin pathway.
- Figure 2 presents results showing that CyPep-1 induces a pro-inflammatory response *in vivo*. A) shows the tumor growth *in vivo* at day 17 after implantation of the colorectal cell line CT26 and melanoma cell line B16-F10 treated with control (saline) or CyPep-1 monotherapy. B) CyPep-1 treatment of B16-F10 and CT26 tumor bearing mice induces profound changes in the immune landscape of tumors. Flow cytometry quantification of the

percent of CD45+ leukocytes (gated in live cells) and CD8+ lymphoid cells after vehicle (CT) and CyPep-1. C) CyPep-1 increases inflammatory cytokines in B16-F10 and CT26 tumours. ELISA quantification of the IL2, TNF $\alpha$  and IFN $\gamma$  secreted in the microenvironment of B16-F10 melanoma (upper panels) and CT26 colorectal (lower panels) tumors treated with control vehicle (CT) or CyPep-1 (CyPep-1). Data are reported in pg/ml standardized to excised tumor weight (g) and represented as an average of 5 tumors per group (each dot represents one mouse). All results are shown as mean  $\pm$  SEM (error bars). Statistically significant differences (indicated by asterisks) are calculated compared to control conditions using an unpaired two-tailed Student's t-test (ns= not significant, \*\* =p< 0.005 and \*\*\*=p<0.0005).

Figure 3 shows the results of CyPep-1 monotherapy in one patient with ACC previously treated with Mitotane (standard of care). The patient had metastatic disease in liver, lung and bones. A) CT images from pre-treatment and 6 months of treatment with CyPep-1. Response based on the Evaluation Criteria in Solid Tumours (RECIST) sum of longest diameters (SOD) show a 47% reduction in the sum of diameters of target lesions (Partial Response). B) Liver parameters measured before and during treatment with CyPep-1.

Figure 4 shows the results of CyPep-1 monotherapy in a second patient with ACC previously treated with Mitotane. The patient had metastatic spread to liver, lung, and bone. A) Histopathological examination of biopsy obtained at 6 weeks of treatment with CyPep-1 shows necrosis and immune infiltration. B) RECIST measurements of target lesions during treatment. C) Liver parameters measured before and during treatment with CyPep-1.

Figure 5 presents results showing that in melanoma cells CyPep-1 treatment decreases the expression of target genes Axin2 and Ccn4/Wisp1, and increases the levels of both phosphorylated forms of  $\beta$ -catenin. A) Quantification of cytoplasmic and nuclear levels of  $\beta$ -catenin in melanoma cell line B16-F10 incubated at increasing concentrations of CyPep-1 for 24 hours. B) Quantification of the  $\beta$ -catenin target genes Axin2 and Wnt Induced Secreted protein-1 (Ccn4/Wisp1) by RT-qPCR in melanoma cell line B16-F10 incubated at a concentration of 7.5 $\mu$ M of CyPep-1 for 24 hours. C) Quantification of phosphorylated  $\beta$ -catenin levels (Ser33/37-Thr41 and Ser45-Thr41) in melanoma cell line B16-F10 incubated at increasing concentrations of CyPep-1 for 24 hours. D) Quantification of phosphorylated  $\beta$ -catenin levels (Ser33/37-Thr41 and Ser45-Thr41) in melanoma cell line B16-F10 incubated at a concentration of 7.5 $\mu$ M of CyPep-1 for 24 hours.

Figure 6 presents results showing that CyPep-1 treatment increases the levels of both phosphorylated forms of  $\beta$ -catenin in adrenocortical carcinoma cells. A) Quantification of phosphorylated  $\beta$ -catenin levels (Ser33/37-Thr41 and Ser45-Thr41) in adrenocortical carcinoma cell line H295R incubated at increasing concentrations of CyPep-1 for 24 hours.

- 5 B) Quantification of phosphorylated  $\beta$ -catenin levels (Ser33/37-Thr41 and Ser45-Thr41) in adrenocortical carcinoma cell line H295R incubated at a concentration of 9.375 $\mu$ M of CyPep-1 for 24 hours.

Figure 7 shows the results of CyPep-1 monotherapy in immunodeficient NSG mice

- 10 subcutaneously injected with H295R human adrenocortical carcinoma cells. When tumours reached 250mm<sup>3</sup>, mice were treated intratumorally once a week with 25mg/kg CyPep-1. A) Tumour volume (mm<sup>3</sup>) 0 to 31 days post injection with CyPep-1 or a vehicle control. Days on which the mice were treated with CyPep-1 or a vehicle control are represented by the dashed lines. At day 8, 5 mice per group were euthanized to harvest tumours for RNA  
15 analysis (presented in D). B) Quantification of tumour volume (mm<sup>3</sup>) at day 0 and day 31 post injection with CyPep-1 (CY-101) or a vehicle control. C) A Kaplan–Meier graph of the probability of survival over 31 days after treatment with CyPep-1 or a vehicle control. D) Quantification of the  $\beta$ -catenin target genes Axin2 and Wnt Induced Secreted protein-1 (Ccn4/Wisp1) by RT-qPCR in tumours harvested from euthanized mice 8 days after the first  
20 treatment with CyPep-1 or a vehicle control.

Figure 8 shows imaging of B16-F10 mouse melanoma cells treated with 20 $\mu$ g/ml CyPep-1 for 24 hours. The cells were stained with anti-Axin2 antibodies and DAPI (to stain the nuclei) and imaged using confocal microscopy. A) Images of treated and untreated cells stained  
25 with Axin2 and DAPI, at a scale of 20 $\mu$ m. B) Sections of the images in section A (indicated by the dashed white rectangles in A) at a scale of 5 $\mu$ m. C) Schematic illustrating the differing cytoplasmic distribution of Axin2 (conductin) when inactive (diffuse) or active (condensates). D) Schematic illustrating that CyPep-1 can bind to the RGS domains of Axin2, reversing their aggregation and activating Axin2, leading to Axin2 polymerisation.

- 30  
Figure 9 shows the results of CyPep-1 treatment in combination with the anti-PD-1 antibody Pembrolizumab in one patient with parathyroid carcinoma, previously treated with surgery and radiotherapy (standard of care), and Nivolumab (anti-PD-1 antibody). The patient had metastatic disease, with multiple lesions in different areas of the body. Only one lesion was  
35 injected with CyPep-1. A1) CT imaging of the injected lesion at baseline (indicated by the dashed white circle). A2) CT imaging of the injected lesion three months after treatment with CyPep-1 (indicated by the dashed white circle). A3) Histological imaging of a biopsy from the

injected lesion at baseline, at a scale of 50µm. A4) Histological imaging of a biopsy from the injected lesion three months after treatment with CyPep-1, at a scale of 50µm. A5) CT imaging of the non-injected lesion at baseline (indicated by the white arrow). A6) CT imaging of the non-injected lesion 20 months after the patient began treatment with CyPep-1 (indicated by the white arrow). B) Graphs showing the percentage change in tumour size of the injected and non-injected lesions over 24 months.

Figure 10 presents graphs showing the pharmacokinetic profile of CyPep-1, measured using liquid chromatography (LC)-tandem mass spectrometry (MS).

10

Figure 11 shows the percentage change in tumour size of non-injected target lesions over 18 months for 8 patients with 7 different cancer types. Patients were administered treatment with CyPep-1 for > 6 months either as a monotherapy, in combination with the anti-PD-1 antibody Pembrolizumab (indicated by an asterisk), or as an intrahepatic injection.

15

Figure 12 presents the survival of 6 adrenocortical carcinoma patients whilst receiving CyPep-1 intrahepatic injections, and post-treatment.

### Examples

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#### Example 1

##### Inhibition of $\beta$ -catenin and WISP-1 (CNN4)

CyPep-1, a D-peptide of SEQ ID NO: 1, was prepared by Bachem AG (Switzerland). The effect of CyPep-1 in inhibiting Wnt/ $\beta$ -catenin pathway was evaluated by assessing the expression of WISP-1, a well-described  $\beta$ -catenin downstream target gene, in various cancer cell lines. A downregulation of WISP-1 indicates decreased transcriptional activity of  $\beta$ -catenin of CyPep-1-treated cells.

In brief, the cell lines were cultured in the presence of CyPep-1 added in the culture media at 0-5 µM for 24h. Control cells were cultured in the medium without CyPep-1 (in PBS). Cells were washed with PBS and lysed using an appropriate cell fractionation kit to recover the cytoplasmic and nuclear fractions according to the supplier instructions. Cytoplasmic and nuclear fractions were separated on an SDS-PAGE gels and proteins were transferred onto nitrocellulose membranes incubated with anti- $\beta$ -catenin antibody and either anti- $\alpha$ -tubulin antibody (a specific cytoplasmic protein) or anti-histone H3 antibody (a specific nuclear protein). The quantification of cytoplasmic and nuclear  $\beta$ -catenin bands was performed by Image J. The ratio  $\beta$ -catenin/ $\alpha$ -tubulin corresponds to the cytoplasmic fraction

of  $\beta$ -catenin, and the ratio  $\beta$ -catenin/histone H3 (Y1) or  $\beta$ -catenin/lamin A/C (B16-F10 and CT26) corresponds to the nuclear fraction of  $\beta$ -catenin. The results are reported as a fold change of the cytoplasmic and nuclear fraction of  $\beta$ -catenin in cells treated with the indicated concentration of CyPep-1 compared to untreated control cells (Figure 1A). To assess the mRNA expression of CyPep-1, melanoma (B16-F10 and Yumm1.7), colorectal cancer (CT26), or glioma (GL261) were treated with CyPep-1 as described above. For CT26 cells, different doses of CyPep-1 were used to assess the dose-dependent decrease of WISP-1 expression.

Figure 1A shows nuclear protein levels of  $\beta$ -catenin in the adrenocortical cell line Y1, colorectal cell line CT26 and melanoma cell line B16-F10 treated with CyPep-1 or control. Results are reported as fold change compared to control. Figures 1B and C show the mRNA expression levels of WISP-1/CCN4 in melanoma (B16-F10), colorectal cancer (CT26), glioma (GL261) and melanoma (Yumm1.7) cell lines treated with CyPep-1. Results are reported as fold change of WISP-1 in treated cells compared to control. In all cell lines, treatment with CyPep-1 significantly reduced the mRNA expression of WISP-1/CCN4.

## Example 2

### CyPep-1 induces a pro-inflammatory response in-vivo

B16-F10 and CT26 cell lines were obtained from ATCC. RPMI 1640, DMEM, FBS, and antibiotics were obtained from Life Technologies. B16-F10 and CT26 cells were cultured in DMEM and RPMI 1640, respectively, supplemented with 10% Fetal Bovine Serum (FBS) and 1% Penicillin/Streptomycin at 37°C and 5% CO<sub>2</sub>. The cell lines were mycoplasma-free based on tests with a Mycoalert kit (Lonza). C57BL/6 and BALB/C (7 weeks old) were obtained from Janvier and housed in pathogen-free conditions for one week prior to the experiments. At day 0, mice were injected subcutaneously in the right flank with  $0.2 \times 10^6$  B16-F10 or  $10^6$  CT26 cells diluted in 100  $\mu$ l of PBS. When median tumour volume reached 100 mm<sup>3</sup>, CyPep-1 was administered 2 mg/kg intra-tumourally or vehicle (PBS).

Tumor weight results are reported as the average of 10 mice per group from 2 independent experiments conducted with 5 mice per group.

Figure 2A shows that in the case of both B16-F10 and CT26 tumour bearing mice, CyPep-1 reduced tumour weight compared to control. Results are shown as mean  $\pm$  SEM (error bars). Statistically significant differences (indicated by asterisks) are calculated using an unpaired two-tailed Student's t-test.

Figure 2B shows that CyPep-1 treatment of B16-F10 and CT26 tumour bearing mice induces profound changes in the immune landscape of tumours. Tumours were harvested and mechanically dissociated into fragments (<4mm), and then enzymatically digested using

mouse tumour dissociation kit (Miltenyi Biotec) for 45 min at 37°C. Single-cell suspensions were prepared and red blood cells were lysed by ACK (10-548E, Lonza).

Cells were counted using a Countess Automated Cell Counter (Invitrogen) and blocked for 30 minutes on ice with Fc block (TruStain fcX™ (anti-mouse CD16/32) Antibody 101320 BioLegend). The following antibodies were used (BioLegend): FITC anti-mouse CD45, APC anti-mouse CD8a, APC/Fire 750 anti-mouse CD4, LIVE/DEAD Fixable Blue Dead Cell Stain Kit (Thermo Fisher Scientific) was used as a viability dye. For compensation controls, single dye stains were performed and fluorescence spread was checked by carrying out FMO controls. The level of non-specific binding was evaluated on isotype controls.

Figure 2C shows that CyPep-1 increases inflammatory cytokines in B16-F10 and CT26 tumours. ELISA quantification of the IL2, TNFα and IFNγ secreted in the microenvironment of B16-F10 melanoma (upper panels) and CT26 colorectal (lower panels) tumours treated with control vehicle (CT) or CyPep-1 (CyPep-1). Data are reported in pg/ml standardized to excised tumour weight (g) and represented as an average of 5 tumours per group (each dot represents one mouse). IL2, TNFα and IFNγ were quantified using mouse IL2 (ref DY410-05), TNFα (ref DY410-05) and IFNγ (ref DY485-05) DuoSet ELISA kits from R&D systems according to manufacturer's protocol.

### Example 3

#### Clinical studies

##### *Materials*

The CyPep-1 peptide was synthesised by Bachem AG (Switzerland). CyPep-1 is an all D-amino acid peptide consisting of the amino acid sequence set forth in SEQ ID NO: 1.

##### *Methods*

The clinical trial assessed CyPep-1 monotherapy. The patient population comprised all-comers enrolled in a basket trial (wherein all solid tumour types are enrolled into the study) with advanced disease (stage IV metastatic disease, wherein most patients exhibited stage IVC disease), wherein all other available treatment options have been exhausted.

Patients received 20 mg CyPep-1 injections every other week (biweekly). Patient responses are defined as either an objective response according to RECIST1.1 guidelines, or if stable disease was present for >16 weeks. RECIST1.1 measures for the evaluation of target lesions include:

- Complete response (CR): Disappearance of all target lesions;

- Partial response (PR): At least a 30% decrease in the sum of the LD target lesions, taking as reference the baseline sum LD;
- Progressive disease (PD): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions;
- Stable disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started.

RECIST1.1 measures for the evaluation of non-target lesions include:

- Complete response (CR): Disappearance of all non-target lesions and normalisation of tumour marker level;
- Stable disease (SD): Persistence of one or more non-target lesion(s) or/and maintenance of tumour marker level above the normal limits;
- Progressive disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions.

### Results

Administration of CyPep-1 monotherapy showed promising results in advanced stage IVC cancers associated with an aberrant activated Wnt/ $\beta$ -catenin pathway:

#### CyPep-1 monotherapy

Figure 3 shows the results of CyPep-1 monotherapy in a patient with ACC. A) shows CT scans of ACC in the patient at baseline and at 6 months of CyPep-1 treatment. Response Evaluation Criteria in Solid Tumours (RECIST) showed a partial response (47%) at 6 months. B) Evaluation of liver enzymes during treatment shows normalization of values.

Figure 4 shows the results of CyPep-1 monotherapy in another patient with ACC. A) histological H&E staining of biopsy showing widespread cell death after treatment with CyPep-1 monotherapy. B) Target lesions in lung and liver during 6 months of treatment. Tumour reduction is observed not only in the injected tumour, but also in the other non-injected metastasis of the liver (same organ of the injected lesions) and lung (distant organ of the injected lesions). The reduction of the non-injected metastasis in distant organs is considered the strongest evidence of an immune-mediated abscopal effect of the intratumorally administered drug. C) Evaluation of liver enzymes during treatment.

Example 4CyPep-1 induces phosphorylation of  $\beta$ -catenin

Melanoma (B16-F10) and adrenocortical carcinoma (H295R) cells were plated. The  
5 B16-F10 cells were treated with increasing concentrations of CyPep-1 (0-7.5 $\mu$ M) after 24  
hours; the H295R cells were treated with increasing concentrations of CyPep-1 (0-9.375 $\mu$ M)  
when 80% confluency was reached. Both cell lines were treated with CyPep-1 for 24 hours,  
and control cells were cultured in the medium without CyPep-1 (in PBS). Cells were then  
harvested and washed with PBS. RIPA buffer (including phosphatase and protease  
10 inhibitors) was used to lyse the cells and extract protein and mRNA. An appropriate cell  
fractionation kit was also used for the B16-F10 cells to recover the cytoplasmic and nuclear  
fractions according to the supplier instructions.

The extracted proteins were separated on an SDS-PAGE gels and transferred onto  
nitrocellulose membranes incubated with: anti- $\beta$ -catenin antibody and either anti- $\alpha$ -tubulin  
15 antibody (a specific cytoplasmic protein) or anti-histone H3 antibody (a specific nuclear  
protein) (Figure 5A); either anti-phospho- $\beta$ -catenin (Ser33/37-Thr41) antibody or anti-  
phospho- $\beta$ -catenin (Ser45-Thr41) antibody and anti-actin antibody (control) (Figures 5C and  
6A). The quantification of phospho- $\beta$ -catenin bands was performed by Image J. The ratio of  
the protein expression of phospho- $\beta$ -catenin/actin is reported as a fold change of the  
20 phospho- $\beta$ -catenin and actin in cells treated with 7.5 $\mu$ M (B16-F10) or 9.375 $\mu$ M (H295R)  
CyPep-1 compared to untreated control cells (Figures 5D and 6B).

Figure 5A shows that with increasing concentrations of CyPep-1, the nuclear fraction  
of  $\beta$ -catenin is decreased, and the cytoplasmic fraction is increased.

Figures 5D and 6B show that in melanoma and adrenocortical carcinoma cells, both  
25 phosphorylated forms of phospho- $\beta$ -catenin are increased (~2 times more) upon treatment  
with CyPep-1. Phosphorylation of  $\beta$ -catenin at the GSK3 (pS33, pS37, and pT41) and CKI  
(pS45) target residues target  $\beta$ -catenin for ubiquitination and subsequent proteasomal  
degradation. These results illustrate that CyPep-1 increases the phosphorylation of these  
residues, and increases the degradation of  $\beta$ -catenin.

30 To assess the mRNA expression of CyPep-1, melanoma (B16-F10) cells were  
treated with CyPep-1 as described above. RT-qPCR was performed to evaluate the mRNA  
expression levels of the Wnt/ $\beta$ -catenin downstream target genes Axin2 and Ccn4/Wisp1.  
The ratio of the mRNA expression of the target gene is reported as a fold change of target  
gene mRNA in cells treated with 7.5 $\mu$ M CyPep-1 compared to untreated control cells (Figure  
35 5B).

Figure 5B shows that CyPep-1 decreases the expression of the Wnt/ $\beta$ -catenin downstream target genes Axin2 and Ccn4/Wisp1 in treated cells compared to the untreated control cells.

#### 5 Example 5

##### In vivo adrenocortical carcinoma study of CyPep-1

- Human adrenocortical carcinoma cells (H295R cell line) were injected subcutaneously into immunodeficient NGS mice. When tumours reached 250mm<sup>3</sup>, mice were treated intratumorally once a week with 25/mg/kg CyPep-1, or a vehicle control. 5 mice per group were euthanized 8 days after the first treatment to harvest the tumours for mRNA analysis. The other animals were treated further, and used for tumour growth (Figure 7A and 7B) and survival analysis (Figure 7C). When tumour volume reaches 1500mm<sup>3</sup>, the mice were euthanized.
- Figures 7A and 7B show that after the first treatment, CyPep-1 significantly reduced tumour volume compared to the vehicle until the end of the experiment. CyPep-1 treated mice appeared tumour-free at the end of the experiment. Figure 7C shows that CyPep-1 was also able to significantly ( $p=0.0062$ ) increase the survival of mice compared to vehicle-treated mice.
- mRNA was extracted from the tumours harvested from mice euthanized at day 8. RT-qPCR was performed to evaluate the mRNA expression levels of the Wnt/ $\beta$ -catenin downstream target genes Axin2 and Ccn4/Wisp1. The ratio of the mRNA expression of the target gene is reported as a fold change of target gene mRNA in tumours treated with CyPep-1 compared to untreated control tumours (Figure 7D).
- Figure 7D shows that CyPep-1 decreases the expression of the Wnt/ $\beta$ -catenin downstream target genes Axin2 and Ccn4/Wisp1 in the treated mouse tumours compared to the untreated control tumours. For Ccn4/Wisp1, this reduction was significant ( $p=0.0083$ ).

#### Example 6

##### 30 Confocal microscopy of Axin2

- Melanoma cells (B16-F10) were seeded in ibidi plates. After 24 hours, the cells were treated with increasing concentrations of CyPep-1 (0-20 $\mu$ g/ml) for 24 hours. The cells were then fixed with 4% PFA and stained with rabbit anti-Axin2 antibody. An anti-rabbit secondary antibody coupled with AlexaFluor633 was used. DAPI was used to stain the nuclei. Samples were observed using a confocal microscope (Figures 8A and 8B).

Figures 8A and 8B show diffuse staining of Axin2 in the untreated sample; this observation is indicative of inactive Axin2. When inactive, the RGS domains of Axin2 aggregate (Figure 8D), and Axin2 presents a diffuse distribution in the cytoplasm (Figure 8C). Figures 8A and 8B also show that condensates/punctates are forming in the majority of the cells treated with CyPep-1; this observation is indicative of activated Axin2. When active, the RGS domains do not aggregate, and Axin2 polymerises (condensates) (Figure 8C). This observation indicates that Axin2 polymerises upon treatment with CyPep-1, suggesting that CyPep-1 activates Axin2. It is believed that CyPep-1 binds to the RGS domains of Axin2 to reverse their aggregation, activating Axin2 (Figure 8D).

### Example 7

#### Clinical studies

##### *Materials*

The CyPep-1 peptide was synthesised by Bachem AG (Switzerland). CyPep-1 is an all D-amino acid peptide consisting of the amino acid sequence set forth in SEQ ID NO: 1.

##### *Methods*

Following the clinical trial described in Example 3, the recommended dose for further development (RP2D = 20mg) was explored across three expansion cohorts: A) CyPep-1 monotherapy, patients received 20 mg CyPep-1 injections every other week (biweekly); B) CyPep-1 and anti-PD-1 antibody, patients received 20 mg CyPep-1 injections every other week (biweekly) and 400 mg pembrolizumab every 6 weeks; C) Intrahepatic CyPep-1 monotherapy, patients received 20 mg CyPep-1 injections every other week (biweekly) in intrahepatic lesions. Response assessment was performed every 8 weeks. Patient responses are defined as either an objective response according to RECIST1.1 guidelines, or if stable disease was present for >16 weeks. RECIST1.1 measures for the evaluation of target lesions are described in Example 3.

##### *Results*

Administration of CyPep-1 monotherapy showed promising results in advanced stage IV cancers associated with an aberrant activated Wnt/ $\beta$ -catenin pathway:

#### CyPep-1 and anti-PD-1 antibody

Figure 9 shows the results of CyPep-1 combination therapy with anti-PD-1 antibody (Pembrolizumab) in a patient with parathyroid carcinoma. A1-2) shows CT scans of the

lesion injected with CyPep-1 at baseline and 3 months after treatment. A3-4) shows histological images of a biopsy from the injected lesion at baseline and 3 months after treatment. The tissue at baseline is indicative of malignancy, however the tissue after 3 months after treatment is indicative of benign tissue. A5-6) shows CT scans of a non-injected lesion at baseline and 20 months after treatment with CyPep-1 began in the injected lesion. The tumour has visibly reduced in size. B) Evaluation of the percentage change in tumour size of the injected and non-injected lesion shows a reduction in size of both tumours over 24 months.

These results illustrate the efficacy of CyPep-1 when injected directly into lesions, but also provides strong evidence of an immune-mediated abscopal effect of the intratumorally administered drug due to the reduction of the non-injected metastasis in distant organs.

#### Pharmacokinetics of CyPep-1

Figure 10 illustrates the favourable pharmacokinetic profile of CyPep-1. The serum concentration reached peak levels  $\approx 15$  minutes after CyPep-1 administration. The lower limit of quantification (LLOQ) was 10ng/mL.

#### Tumour growth kinetics of non-injected target lesions

Figure 11 shows the percentage change in tumour size of non-injected target lesions over 24 months for 8 patients with various cancer types. Patients were administered treatment with CyPep-1 for > 6 months.

Patient details are as follows: sarcoma, CyPep-1 monotherapy, stable disease (SD); chondrosarcoma, CyPep-1 monotherapy, stable disease (SD); uveal melanoma, intrahepatic injections, progressive disease (PD); melanoma, CyPep-1 monotherapy, stable disease (SD); adrenocortical carcinoma, intrahepatic injection, partial response (PR); head and neck squamous cell carcinoma, CyPep-1 and anti-PD-1 antibody, stable disease (SD); adrenocortical carcinoma, intrahepatic injection, stable disease (SD); parathyroid carcinoma, CyPep-1 and anti-PD-1 antibody, unconfirmed progressive disease (UPD); melanoma, CyPep-1 and anti-PD-1 antibody, partial response (PR).

These results provide strong evidence of an immune-mediated abscopal effect of CyPep-1 due to the reduction of the non-injected metastasis in distant organs. This effect is observed for multiple cancer types, and for multiple modes of administration.

Intrahepatic injection

Figure 12 shows the survival of 6 adrenocortical carcinoma patients receiving CyPep-1 intrahepatic injections.

5 Patient details are shown in Table 2 below:

Table 2

| Patient # | iRECIST | Tumour Stage | Metastatic deposits                                             | $\beta$ -catenin (IHC) | Mutations in the $\beta$ -catenin pathway | Mutations in Axin2 network |
|-----------|---------|--------------|-----------------------------------------------------------------|------------------------|-------------------------------------------|----------------------------|
| 10-010    | PR      | IV           | liver, bone                                                     | cytoplasmic            | Axin2, DVL1, APC2, LRP5                   | Yes                        |
| 04-006    | SD      | IV           | liver, lung, bone                                               | nuclear/perinuclear    | GNAI3, MED12                              | Yes                        |
| 10-019    | SD      | IV           | liver, lung, lymph node                                         | nuclear/perinuclear    | CTNNB1, LRP6                              | No                         |
| 10-021    | PD      | IV           | liver, abdominal cavity, lymph node                             | cytoplasmic            | LRP6                                      | No                         |
| 10-022    | PD      | IV           | liver, lung, soft tissue, peritoneum, lymph node, adrenal gland | cytoplasmic            | None                                      | No                         |
| 04-007    | PD      | IV           | liver, lung, adrenal gland                                      | cytoplasmic            | None                                      | No                         |

PR = partial response, SD = stable disease, PD = progressive disease

10 PR and SD indicate disease control (i.e. effective management of the disease)

15 These results show that patients with mutations in the  $\beta$ -catenin pathway, particularly if the gene encodes a protein involved in the Axin2 network, are positively associated with a better iRECIST response and longer survival. This illustrates that patients with mutations in the  $\beta$ -catenin pathway, resulting in aberrant Wnt/ $\beta$ -catenin signalling, respond the best to CyPep-1 treatment. Therefore, mutations in the  $\beta$ -catenin pathway can serve as biomarkers for patients suitable for treatment with CyPep-1.

In this regard, it is noted that patient 10-022 who did not exhibit disease control (had disease progression) was suffering from very advanced disease, and was brought into the

trial at a late stage; the negative response from this one patient is accordingly not indicative that LRP6 cannot serve as a biomarker, and that CyPep-1 therapy is generally not effective, but rather indicates that as response may not be seen in each and every patient, and at every stage of disease. This patient may be regarded as an outlier.

Claims

1. An oligopeptidic compound comprising a D-amino acid sequence as set forth in SEQ ID NO: 1, or a D-amino acid sequence having at least 85 % sequence identity thereto, for use in the treatment of cancer in a subject, wherein said cancer is associated with aberrant activation of the Wnt/ $\beta$ -catenin pathway.
2. The oligopeptidic compound for use according to claim 1, wherein said oligopeptidic compound comprises or consists of the D-amino acid sequence set forth in SEQ ID NO: 1.
3. The oligopeptidic compound for use according to claim 1 or 2, wherein said subject is a mammal, preferably a mouse, rat, guinea pig, cat, dog, pig, horse, cow, sheep, goat, monkey or ape.
4. The oligopeptidic compound for use according to claim 3, wherein said subject is a human.
5. The oligopeptidic compound for use according to claim 4, wherein said cancer is selected from eye cancer, vulvar cancer, endocrine cancer, anal cancer, pancreatic cancer, colorectal cancer, bile duct cancer, liver cancer, kidney cancer, gallbladder cancer, bladder cancer, skin cancer, prostate cancer, breast cancer, head and neck cell cancer, parotid cancer, cervical cancer, oesophageal cell cancer, endometrial cancer, lung cancer, adrenocortical cancer, glioma, ovarian cancer, Wilms' tumour, neuroendocrine (carcinoid) cancer, HPV positive cancer, squamous cell carcinoma and sarcoma, or a desmoid tumour.
6. The oligopeptidic compound of claim 5, wherein said cancer is selected from melanoma, parathyroid cancer, thyroid cancer, head and neck squamous cell cancer (HNSCC), squamous cell cancer of the oesophagus, cervix, vulva, or lung, renal cell carcinoma, small cell lung cancer, non-small cell lung cancer, colon cancer, or rectal cancer.
7. The oligopeptidic compound for use according to claim 6, wherein said cancer is adrenocortical carcinoma.
8. An oligopeptidic compound for use according to any one of claims 1 to 7, wherein said compound is used with a second therapeutic agent effective to treat said cancer.

9. The oligopeptidic compound for use according to claim 8, wherein said second therapeutic agent is selected from a chemotherapeutic agent, immunotherapeutic agent, hormone therapy, radiation therapy or photodynamic therapy.

5 10. The oligopeptidic compound for use according to any one of claims 8 or 9, wherein said second therapeutic agent is a cytotoxic agent, a cell for adoptive cell transfer therapy, an antibody, a hormone, or a checkpoint inhibitor.

11. The oligopeptidic compound for use according to claim 10, wherein said checkpoint  
10 inhibitor blocks the interaction between PD-1 and PD-L1, or between CTLA-4 and CD80 or CD86.

12. The oligopeptidic compound for use according to claim 11, wherein said checkpoint inhibitor is an antibody which binds PD-1, PD-L1, or CTLA-4.

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13. The oligopeptidic compound for use according to any one of claims 1 to 12, wherein said compound is formulated for intra-tumoural injection or infusion.

14. The oligopeptidic compound for use according to any one of claims 1 to 13, wherein  
20 said compound is for local administration to a primary or secondary tumour.

15. The oligopeptidic compound for use according to claim 14, wherein said compound causes regression of primary and secondary tumours in the subject.

25 16. A method of treating a cancer associated with aberrant activation of the Wnt/ $\beta$ -catenin pathway, said method comprising administering to a subject in need thereof an oligopeptidic compound, optionally together with a second therapeutic agent, wherein said oligopeptidic compound, subject, cancer, treatment, and second therapeutic agent are as defined in any one of claims 1 to 15.

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17. Use of an oligopeptidic compound in the manufacture of a medicament for treating a cancer associated with aberrant activation of the Wnt/ $\beta$ -catenin pathway in a subject, optionally in combination with a second therapeutic agent, wherein said oligopeptidic compound, subject, cancer, treatment, and second therapeutic agent are as defined in any  
35 one of claims 1 to 15.

18. A product comprising an oligopeptidic compound as defined in claim 1 or 2 and a second oncotherapeutic agent as a combined preparation for separate, simultaneous or sequential use in the treatment of cancer in a subject, wherein said cancer is associated with aberrant activation of the Wnt/ $\beta$ -catenin pathway.

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19. The product of claim 18, wherein the oligopeptidic compound, subject, cancer, and second therapeutic agent are as defined in any one of claims 2 to 15.

20. A method of identifying a subject for treatment of a cancer with an oligopeptidic compound according to any one of claims 1 to 15, said method comprising determining, in a sample obtained from a subject, the presence of a biomarker indicative of an activated Wnt/ $\beta$ -catenin pathway.

21. The method of claim 20, wherein said biomarker is:

15 (i) an increase in expression of one or more of the following proteins in a tumour sample:  $\beta$ -catenin, WISP-1, Axin2, FZD3, FZD6, FZD7, Wnt 3, Wnt4, Wnt 5A, RSPO1, RSPO2, RSPO3, RSPO4, c-MYC, cyclin-D, TCF21;

(ii) a mutation in one or more genes selected from:  $\beta$ -catenin (CTNNB1), AXIN1, AXIN2, APC, APC2, ZNRF3, MEN1, GNAI2, RNF43, DVL1, LPR5, LPR6, MED12, and

20 BCL9L;

(iii) upregulation of expression of one or more of the following genes: WISP-1, Axin2, c-MYC, cyclin-D, TCF21, FZD3, FZD6, FZD7, Wnt 3, Wnt4, Wnt 5A, RSPO2, CLDN1, LGR5.

22. The method of claim 20 or claim 21, wherein said biomarker is a mutated gene encoding a protein involved in the Axin2 regulatory network, wherein the gene contains one or more mutations.

23. The method of any one of claims 20 to 22, wherein said gene is selected from:

30 APC2, AXIN2, DVL1, LRP5, MEN1, GNAI3, MED12, CTNNB1, LRP6.

24. A method of identifying and treating cancer associated with aberrant activation of the Wnt/ $\beta$ -catenin pathway in a subject, said method comprising:

(i) identifying a subject for treatment of the cancer by determining, in a sample

35 obtained from the subject, the presence of a biomarker indicative of an activated Wnt/ $\beta$ -catenin pathway, wherein said identifying is performed by a method as defined in any one of claims 20 to 23; and

(ii) administering to said subject an oligopeptidic compound comprising a D-amino acid sequence as set forth in SEQ ID NO: 1, or a D-amino acid sequence having at least 85 % sequence identity thereto, optionally wherein every amino acid of the compound is a D-amino acid, optionally together with a second therapeutic agent.

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