*TOPIC HIGHLIGHT*

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# Involvement of substance P and the NK-1 receptor in pancreatic cancer

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## Abstract

Pancreatic cancer is the fourth leading cause of cancer related-death for both men and women and the 1- and 5-year relative survival rates are 25% and 6%, respectively. Thus, it is urgent to investigate new antitumor drugs to improve the survival of pancreatic cancer patients. The peptide substance P (SP) has a widespread distribution throughout the body. After binding to the neurokinin-1 (NK-1) receptor, SP regulates biological functions related to cancer, such as tumor cell proliferation, neoangiogenesis, the migration of tumor cells for invasion, infiltration and metastasis, and it exerts an antiapoptotic effects on tumor cells. It is known that the SP/NK-1 receptor system is involved in pancreatic cancer progression: (1) pancreatic cancer cells and samples express NK-1 receptors; (2) the NK-1 receptor is overexpressed in pancreatic cancer cells in comparison with non-tumor cells; (3) nanomolar concentrations of SP induce pancreatic cancer cell proliferation; (4) NK-1 receptor antagonists inhibit pancreatic cell proliferation in a concentration-dependent manner, at a certain concentration, these antagonists inhibit 100% of tumor cells; (5) this antitumor action is mediated through the NK-1 receptor, and tumor cells die by apoptosis; and (6) NK-1 receptor antagonists inhibit angiogenesis in pancreatic cancer xenografts. All these data suggest that the SP/NK-1 receptor system could play an important role in the development of pancreatic cancer; that the NK-1 receptor could be a new promising therapeutic target in pancreatic cancer, and that NK-1 receptor antagonists could improve the treatment of pancreatic cancer.

**Core tip:** The substance P (SP)/neurokinin-1 (NK-1) receptor system plays an important role in pancreatic cancer progression. Pancreatic cancer cells overexpress NK-1 receptors and SP promotes angiogenesis and the proliferation and the migration of pancreatic tumor cells. By contrast, NK-1 receptor antagonists, in a concentration-dependent manner, inhibit pancreatic cell proliferation (tumor cells die by apoptosis), have antiangiogenic properties in pancreatic cancer and block the migratory activity of pancreatic tumor cells. The antitumor action is mediated through the NK-1 receptor. Thus, the NK-1 receptor could be a new promising therapeutic target in pancreatic cancer and NK-1 receptor antagonists could improve pancreatic cancer treatment.

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# INTRODUCTION

Pancreatic cancer is the fourth leading cause of cancer related-death for both men and women, with less than 5% survival at 5 years after diagnosis. In 2013, the American Cancer Society estimated 45220 new cases of pancreatic cancer in the United States and 38460 deaths from the disease. Treatment strategies have not succeeded in significantly extending patient survival, and neither have clinical outcomes improved substantially over the past 35 years; the overall 5-year survival rate remains dismal, at around 5%[1]. Pancreatic cancer remains a major unsolved health problem and conventional treatments having little impact on the course of the disease. Moreover, almost all patients with pancreatic cancer develop metastases, this being the primary reason for its lethality[2]. Accordingly, there is an urgent need to improve current therapies. Cytostatic drugs show a low safety profile and severe side effects, since they are not specific to tumor cells. Research should focus on drugs with the same or greater antitumor action but with fewer side effects. This can only be achieved if the drug is specific against pancreatic cancer cells and researchers are therefore seeking to identify novel molecular targets for blocking pancreatic cancer growth.

For some years, the expression and secretion of peptides by tumors has attracted increasing interest[3]. Substance P (SP) is an undecapeptide that is widely distributed throughout the body. It is derived from the pre-protachykinin A gene and belongs to the tachykinin family of peptides. The biological actions of tachykinins (SP, neurokinin A, neurokinin B...) are mediated through the neurokinin-1 (NK-1), NK-2 and NK-3 receptors. SP has the highest affinity for the NK-1 receptor, which shows a widespread distribution throughout the body. This means that the biological actions (*e.g.*, pain, neurogenic inflammation, regulation of the cardiovascular system, mitogenesis...) exerted by the SP are mainly mediated by the NK-1 receptor[4,5]. Moreover, there are many data suggesting the involvement of the SP/NK-1 receptor system in cancer[5] (Figure 1 and Table 1). SP and NK-1 receptors have been detected in tumor cells and in intra- and peri-tumoral blood vessels[4-6]. SP induces mitogenesis in normal and tumor cells, protecting the latter from apoptosis, and controls the migration of tumor cells[4,7,8]. This is extremely important since the prevention of metastasis is a major goal in the treatment of tumors because over 90% of cancer deaths are derived not from the primary tumor but from the development of metastases. Moreover, it has recently been reported that the extravasation of tumor cells into the brain to form cerebral metastases may be an SP-mediated process[9]. More specifically, it has been reported that the SP/NK-1 receptor system is involved in pancreatic cancer by inducing pancreatic cancer proliferation, neoangiogenesis, and migration of pancreatic cancer cells (invasion, infiltration and metastasis). By contrast, NK-1 receptor antagonists inhibit pancreatic cancer cell proliferation (tumor cells die by apoptosis), angiogenesis and the migration of pancreatic cancer

| Table 1 Technical features of NK-1 receptor antagonists | |
|---|---|
| **NK-1 receptor antagonists** | **Feature** |
| Therapeutic action | Linked to stereochemical features (receptor affinity) and not to chemical composition |
| Cell specificity | Specific cytotoxicity against pancreatic cancer cells *via* the NK-1 receptor |
| Antitumor action | Mitogenesis inhibition |
| | Cell death by apoptosis |
| | Angiogenesis inhibition |
| | Inhibition of the migration of cancer cells: Inhibit invasion, infiltration and metastasis |
| Beneficial effects | Central nervous system: |
| | Antiemetic |
| | Anxiolytic |
| | Antimigraine |
| | Anticonvulsant |
| | Neuroprotector |
| | Peripheral nervous system: |
| | Neuroprotector |
| | Liver: |
| | Hepatoprotector |
| | Kidney: |
| | Nephroprotector |
| | Systemic: |
| | Analgesic |
| | Antiinflammatory |
| | Antiviral |
| Side-effects | Headaches, hiccupping, vertigo and drowsiness |
| Synergistic effect with cytostatic and radiation therapy | Vinblastine, adriamycin, mitomycin, ifosfamide, cisplatin |
| Decrease cytostatic and radiation therapy side-effects | Cisplatin, cyclophosphamide |
| Block multiple intracellular signaling pathways | NK-1 receptor (G protein-coupled receptor): Rho-Rock-pMLC: Cell migration inhibition |
| | PLC-IP$_3$-Akt: Apoptotic effect |
| | PLC-DAG-TK-MAPKs: Inhibition of tumor cell proliferation |
| | PLC-DAG-PKC-MAPKs: Inhibition of tumor cell proliferation |
| | ATP-cAMP-PKA-Phosphorylation |
| | PLA-Arachidonic acid-PGs |
| | TXAs |
| | LXs |
| | Glycogen breakdown inhibition (counteract the Warburg effect) |
| Dosage | Act at μmol/L in a concentration-dependent manner |

Akt: Protein kinase B; ATP: Adenosine triphosphate; cAMP: Cyclic adenosine monophosphate; DAG: Diacilglicerol; IP3: Inositol triphosphate; LXs: Leukotrienes; MAPKs: Mitogen-activated protein kinase; PGs: Prostacyclin; PKA: Protein kinase A; PKC: Protein kinase C; PLA: Phospholipase A; PLC: Phospholipase C; pMLC: Myosin regulatory light chain phosphorylation; TK: Tyrosine-kinase; TXAs: Thromboxanes.

cells[10-14] (Figure 1 and Table 1).

In sum, all the data reported above suggest that novel possibilities for translational research are emerging to improve the diagnosis and treatment of pancreatic cancer. Here, we review the involvement of the SP/NK-1 receptor system in pancreatic cancer and, specifically, the use of NK-1 receptor antagonists as antitumor drugs in pancreatic cancer (Figure 1 and Table 1).
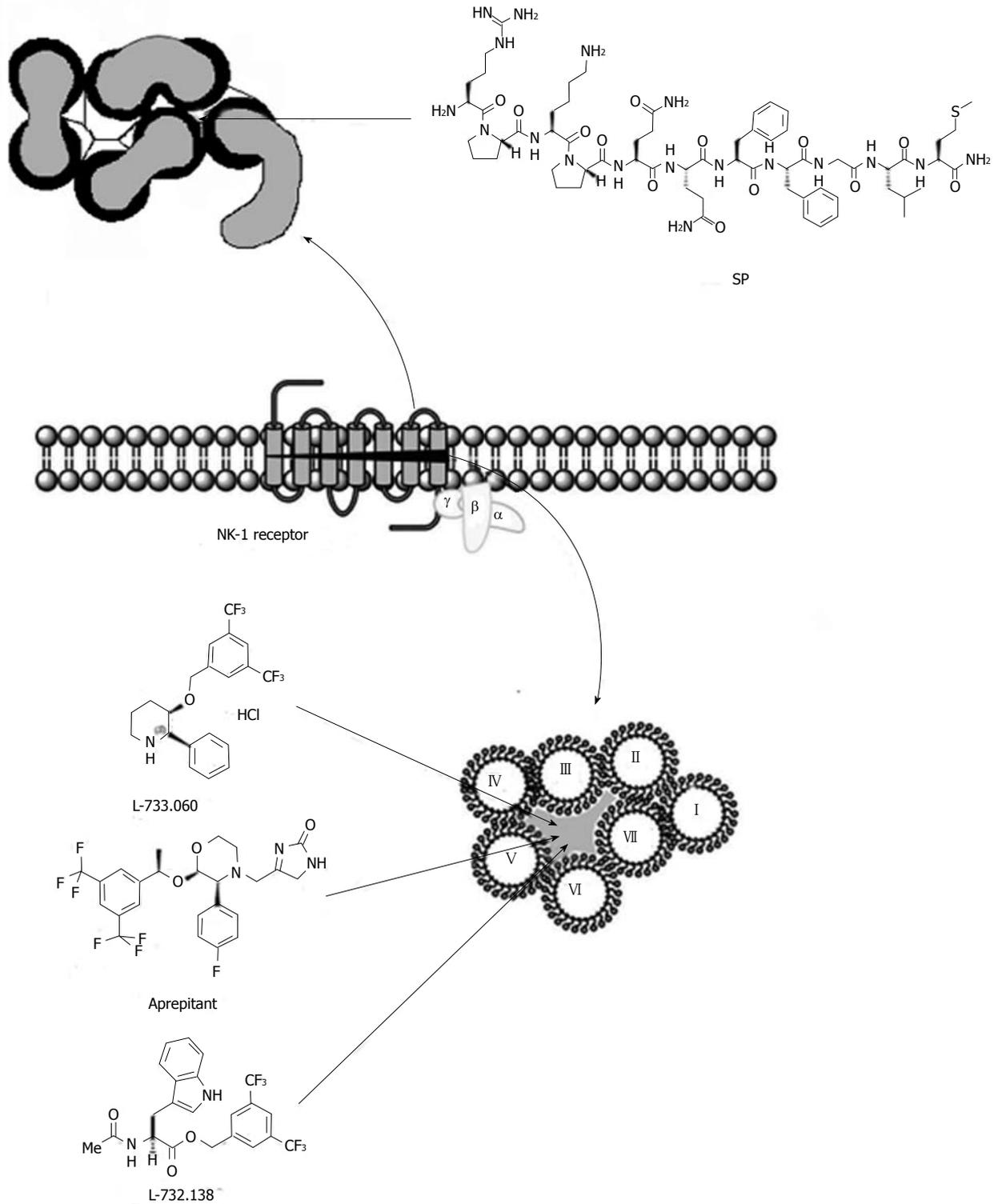
**Figure 1 Substance P and neurokinin-1 receptor antagonists bind to different sites of the neurokinin-1 receptor.** Substance P (SP) binds to the extracellular loops of the receptor, whereas neurokinin-1 (NK-1) antagonists (*e.g.*, L-733.060, aprepitant, L-732.138) bind more deeply, between the transmembrane segments.

## PANCREATIC CANCER CELLS AND SAMPLES EXPRESS NK-1 RECEPTORS

The NK-1 receptor is synonymous with the SP receptor and tachykinin receptor 1. The NK-1 receptor is a G protein-coupled receptor (GPCR) that mediates the action of SP and other tachykinins[15,16]. The NK-1 receptor

consists of 407 amino acid residues; it has a molecular weight of 58 kDa, and it is made of seven hydrophobic transmembrane domains with three extracellular and three intracellular loops, an amino-terminus and a cytoplasmic carboxy-terminus[17,18] (Figure 1). The loops have functional sites, including two cysteines amino acids for a disulfide bridge, Asp-Arg-Tyr, which is responsible for

the association with arrestin and, Lys/Arg-Lys/Arg-X-X-Lys/Arg, which interacts with G-proteins[18,19]. The NK-1 receptor is coupled to the Gq family of G proteins and its activation leads to the hydrolysis of membrane phosphoinositides, resulting in the formation of two-second messengers: inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG)[20,21]. The formation of IP3 triggers the release of calcium from intracellular stores and the formation of DAG leads to the activation of protein kinase C. Together, these messengers cause a cascade of protein phosphorylation/dephosphorylation reactions, culminating in altered gene expression and cell function.

SP is an undecapeptide widely distributed throughout the body and it is the natural ligand showing the highest affinity for the NK-1 receptor (Figure 1). In fact, the NK-1 receptor has been defined as a mediator of the biological activities encoded by the C-terminal sequence of tachykinins, for which SP is a more potent agonist than neurokinin A or neurokinin B[22]. After binding to the NK-1 receptor, SP regulates many biological functions (*e.g.*, pain, neurogenic inflammation, mitogenesis...)[4,5], although other NK receptors could also be involved (*e.g.,* NK-2) in these actions. After the binding of SP to the NK-1 receptor, both are internalized into endosomes; the undecapeptide induces a clathrin-dependent internalization of the receptor, after which SP is degraded and the NK-1 receptor is recycled to the cell surface[23-26]. SP-NK-1 receptor binding can generate second messengers [cyclic adenosine monophosphate (cAMP) accumulation *via* stimulation of adenylate cyclase; stimulation, *via* phospholipase C, of phosphatidyl inositol turnover, leading to calcium mobilization; arachidonic acid mobilization *via* phospholipase A2], triggering numerous effectors mechanisms involved in cellular excitability and in the regulation of cell function[4,5,27].

It is known that pancreatic cancer cells and samples express the NK-1 receptor[10,13,14]. This receptor has been also demonstrated in human cancer cell lines and/or in primary tumors (*e.g.*, glioma, astrocytoma, retinoblastoma, ganglioneuroblastoma, leukemia, neuroblastoma, carcinomas (larynx, gastric, colon, medullary thyroid, breast, oral...)[4-6,10,28-37]. In addition, in most tumors investigated NK-1 receptors have been found in intra- and peritumoral blood vessels. This is quite important regarding the involvement of the NK-1 receptor in angiogenesis[6]. NK-1 receptors have been located in both the plasma membrane and the cytoplasm of tumor cells and, occasionally, in the nucleus of these cells[31,34,38]. Moreover, several isoforms (33-38, 46, 54-58 and 75 kDa) of the NK-1 receptor have been reported in human cancer cells (*e.g.*, neuroblastoma, retinoblastoma, larynx carcinoma, gastric adenocarcinoma, leukemia, *etc.*)[33-36,38]. Regarding the pancreatic cancer, it has been reported that its tumor cells express several isoforms (36, 46, 58 and 75 kDa)[10,13,14]. However, in order to clarify the functional roles of these isoforms, further research is needed. In humans, the presence of two subtypes of the NK-1 receptor has been reported: the full-length one and the truncated one. The former mediates a slow growth of tumor cells and the second enhances the growth of these cells to a considerable extent and stimulates the production of cytokines with growth-promoting functions[39]. It seems that these cytokines activate a transcription factor (NF-κB) that upregulates the truncated NK-1 receptor form and slightly increases the full-length form[40,41]. It is also known that the truncated form, an oncogenic isoform of the NK-1 receptor, mediates malignancy in tumor cells[39] and that the truncated NK-1 receptor is increased in colonic epithelial cells from patients with colitis-associated cancer[42].

In the first study in which NK-1 receptors were reported in pancreatic cancer (1 of 9 samples)[6], the authors applied an autoradiographic method. Later, in another study, NK-1 receptor expression was reported in 27% of the samples[43]. However, a third study compared 50-pancreatic human cancer samples obtained from pancreatoduodenoctomy (Whipple operation) with normal controls[10]. In these cases, the authors found the expression of NK-1 receptors in all the pancreatic cancer samples. Thus, by using *in situ* hybridization and immunohistochemistry techniques, in normal pancreas NK-1 receptor mRNA and NK-1 receptor immunoreactivity were occasionally weakly observed in acinar and ductal cells, but a moderate to strong NK-1 receptor mRNA signal and NK-1 receptor immunoreactivity were present in most of the cancer cells[10]. Moreover, the growth of the tumor mass, peritumoral infiltration and metastasis could be regulated by the SP/NK-1 receptor system, overexpressed in tumor cells and in tumoral and peritumoral tissue in pancreatic cancer (inflammatory cells, fibroblasts, blood vessels, nerves, ganglia, islet)[10].

The NK-1 receptor is also known to be involved in the viability of tumor cells. It has been reported that after a knockdown gene-silencing method (siRNA), the NK-1 receptor is involved in the viability of such cells[33,34,37]. Following the administration of the siRNA *TACR1* (tachykinin 1 receptor gene) to cultured tumor cells, more apoptotic cells were found in siRNA cells than in cells not transfected, and hence the number of siRNA tumor cells was significantly decreased in comparison with the number of non-transfected cells[33,34,37].

## NK-1 RECEPTOR IS OVEREXPRESSED IN PANCREATIC CANCER CELLS IN COMPARISON WITH NON-TUMOR CELLS

It is known not only that the NK-1 receptor is expressed in tumor cells, but also that this receptor is overexpressed in such cells (*e.g.*, glioblastoma, breast cancer, retinoblastoma, larynx, pancreatic, gastric and colon carcinomas...)[4,5,10,30,33,34,37]. This is important, since the visualization of NK-1 receptors by immunohistochemistry for diagnostic or therapeutic purposes would facilitate the identification of tumors overexpressing this receptor[44]. It is known that normal cells express a lower number of NK-1 receptors than tumor cells (*e.g.*, human pancreatic cancer cell lines express more NK-1 receptors than

control cells)[10]; that tumor samples from patients with advanced tumor stages exhibit significantly higher NK-1 receptor levels[10]; that TACR1 mRNA is present in human acute lymphoblastic leukemia cell lines, with the highest levels in these cells and the lowest ones in normal cells[33]; that astrocytoma/glioma cell lines in culture shows a lower number of NK-1 receptors than astrocytoma/glioma primary tumors; that glioblastomas express more NK-1 receptors than astrocytomas, and that the most malignant phenotypes of tumors show a higher rate of NK-1 receptor expression and are associated with advanced tumor stages and a poorer prognosis[6,10,45]. The data suggest that the number of NK-1 receptors could be correlated with the degree of malignancy. Thus, the overexpression of the NK-1 receptor in tumor cells suggests the possibility of finding a specific treatment against cancer using NK-1 receptor antagonists and, in this way, the side effects of the treatment could be decreased considerably. This strategy opens up new approaches for cancer treatment. Moreover, following the use of real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR) methodology in 50 pancreatic human cancer samples obtained from pancreatoduodenoctomy (Whipple operation), NK-1 receptor mRNA levels were increased 36.7-fold in these samples in comparison with normal controls. Enhanced NK-1 receptor expression levels were not related to tumor grade but were associated with advanced tumor stage and a poorer prognosis. As reported above, NK-1 receptor mRNA levels and NK-1 receptor immunoreactivity are higher in human pancreatic cancer samples than in normal pancreas[10]. Moreover, using a Western blot analysis, the NK-1 receptor was found to be increased 26-fold in pancreatic cancer samples in comparison with normal controls. NK-1 receptor mRNA was detected in five pancreatic cancer cell lines by real-time quantitative RT-PCR, the highest levels being observed in CAPAN-1 cells and the lowest ones in ASPC-1 cells. SP and SP analog agonists stimulated pancreatic cancer cell growth, depending on the NK-1 receptor expression level, and this effect could be blocked by a selective NK-1 receptor antagonist in a concentration-dependent manner[10,13].

It has been suggested that chronic inflammation could be correlated with an increased risk of developing cancer. It is known that the risk of pancreatic cancer is very high in subjects with chronic pancreatitis and appears to be independent of sex, country, or type of pancreatitis[46] and that the up-regulation of the NK-1 receptor mRNA expression in chronic pancreatitis has a strong relationship with the pain syndrome that these patients experience[47]. Thus, overexpression of the NK-1 receptor could be involved in chronic pancreatitis-associated cancer. It has also been reported recently that the truncated NK-1 receptor is overexpressed in colonic epithelial cells from patients with colitis-associated cancer, whereas the full-length is not affected[42]. Thus, the overexpression of NK-1 receptors could be used as a diagnostic marker to identify patients at risk of neoplasms and may serve as a useful therapeutic target in the treatment of chronic

inflammation-associated cancer.

## NANOMOLAR CONCENTRATIONS OF SP INDUCE PANCREATIC CANCER CELL PROLIFERATION AND THE MIGRATION OF TUMOR CELLS

SP acts as a mitogen in normal and tumor cells (*e.g.*, neuroblastoma, astrocytoma, melanoma, retinoblastoma, glioma, melanoma, larynx carcinoma, gastric and colon carcinoma, lymphoblastic leukemia) *via* the NK-1 receptor, since the growth inhibition of many human tumor cells after the administration of NK-1 receptor antagonists is partially reversed by the administration of SP[4,5,33-38,48]. Regarding pancreatic cancer cells, nanomolar SP concentrations elicit the proliferation of the pancreatic cancer CAPAN-1, PA-TU 8902, BxPC-3 and MIA PaCa-2 cell lines[13,14]. By contrast, the mitogenic action of SP on these cell lines could be partially reversed by using NK-1 receptor antagonists such as L-733,060, L-732.138 or the drug aprepitant[12-14]. Many data indicate that SP in a universal mitogen in NK-1 receptor-expressing tumor cells. The undecapeptide can be synthesized and secreted by tumor and non-tumor cells and SP can be released from nerve terminal, and/or it can be released into blood vessels[4,5]. Through these paths, the peptide can exert a mitogenic action on tumor cells. The regulation of local tumor activity through sensory nerves containing SP is relevant, since the undecapeptide could modulate the growth of tumor cells, exerting a direct interaction between the nervous system and the tumor cells. Thus, SP could induce mitogenesis *via* the following mechanisms: (1) autocrine (SP is secreted from tumor cells); (2) paracrine (SP exerts a mitogenic action in endothelial cells); (3) SP is released from nerve terminals; (4) SP reaches the whole body through the bloodstream; this is regulated by the limbic system; and (5) endocrine (SP is released from the tumor mass into the blood vessels)[3-5].

There are multiple cell signaling pathways regulated by SP. After the activation of the NK-1 receptor by SP, an increase in DNA synthesis has been reported in tumor cells, and it seems that *via* the NK-1 receptor the undecapeptide activates members of the mitogen-activated protein kinase (MAPK) family, including extracellular signal-regulated kinases 1 and 2 (ERK1/2) and p38MAPK[45] (Table 1). Once activated, ERK1/2 is translocated into the nucleus, inducing proliferation and protecting the cell from apoptosis[5,7]. In tumor cells, SP increases the phosphorylation and activity of Akt or protein kinase B, a serine-threonine protein kinase that becomes activated *via* phosphatidyl-3-kinase (PI3K); the activation of Akt suppresses apoptosis[49,50]. By contrast, NK-1 receptor antagonists inhibit the basal activity of Akt[51] (Table 1). After it has bound to the NK-1 receptor, other effects are also exerted by SP in tumor cells: it activates phospholipase D and enhances forskolin-stimulated cyclic AMP-production; SP induces the release of interleukins, taurine and

glutamate; it mobilizes intracellular calcium; it induces the formation of inositol phosphate; it stimulates glycogen breakdown; and it influences glutamate and $K^+$ transport[5,52-56]. The release of interleukins, taurine and glutamate by tumor cells induces an inflammatory process, increasing the levels of SP and hence increasing tumor cell proliferation. Moreover, it has been reported that after binding to the NK-1 receptor SP stimulates glycogen breakdown and increases the intracellular $Ca^{2+}$ concentration in astrocytoma cells. Both effects occur in a concentration-dependent manner. These effects are completely blocked by the NK-1 receptor antagonist CP-96345[55]. In addition, one of the most prominent metabolic alterations in cancer cells is the increase in aerobic glycolysis and the dependency on the glycolytic pathway for adenosine triphosphate generation, known as the Warburg effect, because most cancer cells predominantly produce energy by means of a high rate of glycolysis followed by lactic acid fermentation[57]. Growing tumor cells typically have glycolytic rates up to 200 times higher than those of their normal tissues of origin; this occurs even if oxygen is plentiful. Thus, after binding to the NK-1 receptors located in tumor cells, SP causes glycogen breakdown and the glucose obtained would be used by tumor cells to increase their metabolism[55]. This mechanism could partly explain the Warburg effect. By contrast, NK-1 receptor antagonists block glycogen breakdown in tumor cells[55], and hence can counteract the Warburg effect[3] (Table 1). This new approach to the NK-1 receptor is very interesting because until now the main goal has been the inhibition of the glycolytic enzymes. However, this strategy has not provided any practical results. In cancer treatment, a reduction in glucose formation by blocking the NK-1 receptor may be possible and indeed easier using NK-1 receptor antagonists. Accordingly, without glucose the Warburg effect is not possible in cancer cells.

The migration of tumor cells is a crucial requirement for the development of metastasis and the progression of cancer. At present, over 90% of cancer deaths are derived not from the primary tumor but from the development of metastases[58]. Thus, a major goal in the treatment of cancer should be to inhibit the development of metastases. In this sense, it is known that tumor cell migration is induced by classical neurotransmitters (dopamine, noradrenalin) and peptides (*e.g.*, SP) and that such migration is inhibited after the administration of $D_2$ receptor, adrenoceptor or NK-1 receptor antagonists[5,59]. It is also known that after binding to the NK-1 receptor SP induces a rapid change in cellular shape (including blebbing) and that membrane blebbing is important in cell movement, cell spreading, and cancer cell infiltration[60,61]. It has recently been reported that SP is involved in pancreatic cancer perineural invasion and that in pancreatic cancer cells SP induces cancer cell proliferation and invasion as well as the expression of matrix metalloproteinase (MMP)-2. SP also promotes neurite outgrowth and the migration of pancreatic cancer cell clusters to the dorsal root ganglia of newborns[14].

# NK-1 RECEPTOR ANTAGONISTS INHIBIT PANCREATIC CELL PROLIFERATION IN A CONCENTRATION-DEPENDENT MANNER

At a certain concentration, these antagonists inhibit 100% of tumor cells. NK-1 receptors antagonists are a broad group of heterogeneous chemical compounds (Figure 1 and Table 1). There are two groups: peptide NK-1 receptor antagonists and non-peptide NK-1 receptor antagonists.

## Peptide NK-1 receptor antagonists
Most of the work carried out on the design and preparation of antagonists of the NK-1 receptor has focused on the introduction of *D*-amino acids[18]. However, their affinity is several orders of magnitude lower than that of natural agonists, and the metabolic instability of peptide NK-1 receptor antagonists and their inability to gain access to the central nervous system through the blood-brain barrier limit their usefulness for *in vivo* studies. In addition, these substances generally have a number of drawbacks, such as poor potency and a lack of the ability to discriminate between tachykinin receptors, partial residual agonist activity, mast cell degranulating activity, and neurotoxicity after administration in the central nervous system[22]. Some of these peptide NK-1 antagonists are[18]: [D-Arg1, D-Trp 7,9, Leu11] SP (Spantide I). This antagonist is neurotoxic and a potent histamine releaser from mast cells; H-D-Lys (Nicotinoyl)-Pro-[3-(3-pyridyl)-Ala]-pro-D-Phe83,4-Cl2)-Asn-DTrp-Phe-D-Trp-Leu-Nle-NH2 (Spantide Ⅱ). This antagonist is devoid of neurotoxicity; [D-Arg1, D-Trp5, 7, 9, Leu11] SP. This antagonist has anticancer effects in a variety of *in vitro* and *in vivo* models (*e.g.*, pancreatic cancer)[11, 62-65]; (D-Arg1, D-Phe5, D-Trp7, 9, Leu11) SP; (D-Arg1, D-Pro2, D-Trp 7,9, Leu11) SP; [Arg6, D-Trp7,9, MePhe8] SP (6-11); [D-Pro2- Trp7, 9] SP; [D-Pro4, D-Trp7, 9, 10, Phe11] SP (4-11); p-HOPA-DTrp-Phe-DTrp-Leu-Leu-NH2: NY-3238; DMePhe-DTrp-Phe-DTrp-Leu(CH2NH)Leu-NH2: NY-3460.

## Non-peptide NK-1 receptor antagonists
Since non-peptide NK-1 receptor antagonists became available[66-68], an increasing number of papers describing new non-peptide antagonists have been published[69]. Thus, steroids (WIN- 51708, *etc.*), perhydroisoindolones (RP-67580, RP-73467, RPR-100.893, *etc.*), benzylamino and benzylether quinuclidine (CP-96345, L-709.210, *etc.*), benzylamino piperidines (CP-99,994, GR-203040, GR-205.171, CP-122.721, *etc.*), benzylether piperidines (L-733.060, L-741.671, L-742.694, *etc.*) and tryptophan based (L-732.138, L-737.488, *etc.*) NK-1 receptor antagonists have been reported[22]. Investigation into non-peptide NK-1 receptor antagonists is a fast-developing field. Some of these peptide NK-1 antagonists have been used in clinical trials and found to be safe. Examples are the drug aprepitant (Figure 1) and its prodrug fosaprepitant, casopitant (GW-679769), vofopitant (GR-205171),

L-759.274, CP-122.721, Ezlopitant (CJ-11.974), Rolapitant, L-754.030, Serlopitant and CJ-11.974[70].

The binding sites for NK-1 receptor antagonists and SP are different[5]. SP is hydrophilic and binds to the extracellular ends of the transmembrane helices and especially to the extracellular loops of the receptor, whereas NK-1 receptor antagonists are lipophilic and bind more deeply between the transmembrane Ⅲ-Ⅶ domains (Figure 1). After binding to the NK- 1 receptor, NK-1 receptor antagonists could block the functions of SP (Table 1). The pharmacologic effect is related to stereochemical features and is not linked to chemical composition. The action is concentration- and time-dependent manner. At higher concentrations, the beneficial effect in the host is summative. Thus, the pharmacologic effects of the NK-1 receptor antagonists are: anxiolytic, antidepressant, antiemetic, antimigraine, antialcohol addiction or neuroprotector effect in the central nervous system, and they also play a role in analgesic, antiinflammatory, and hepatoprotector processes, as well as in antivirus proliferation (Table 1). Regarding cancer, NK-1 receptor antagonists exert an antitumor action (inducing tumor cell death by apoptosis), and they have antiangiogenesis effects and inhibit the migration of tumor cells[3-5] (Table 1). Therefore, the NK-1 receptor antagonists could be considered a new generation of anticancer drugs[3-5,71].

In 1993, Merck initiated studies on NK-1 receptor antagonists based on both CP-96,345 and CP-99,994. L-733.060 (Figure 1) is one of the compounds developed from CP-99,994. It is a 3,5-bistrifluoromethyl benzylether piperidine[72]. The administration of the NK-1 receptor antagonist L-733.060 produces analgesia[73] and antidepressive effects[74,75]. The compound has been suggested for the treatment of anxiety and mood disorders[76] and in inflammatory liver disease, most likely owing to its ability to inhibit the effects of SP[77]. In addition, it has been reported that the NK-1 receptor antagonist L-733.060 acts as an antitumor agent in several human tumor cell lines[13,38,78-81]. In fact, this antitumor action has been reported against pancreatic cancer cell lines[13,14].

A morpholine nucleus that was introduced in the NK-1 receptor antagonist L-742.694 was found to enhance NK-1 receptor-binding affinity[82]. This nucleus was kept in further modifications of the molecule. In order to prevent possible metabolic deactivation, several refinements such as methylation on the C alpha of the benzyl ring and fluorination on the phenyl ring were introduced. These changes afforded the compound MK-869, which showed high affinity for the NK-1 receptor. MK-869 is also called aprepitant (Figure 1) and it has been tested for the treatment of several disorders. Those studies led the Food and Drug Administration to approve the drug Emend, which is indicated for chemotherapy-induced nausea and vomiting and is available for oral use[83]. A water-soluble phosphoryl prodrug for intravenous use, called fosaprepitant, is also available and is marketed as Ivemend[84]. It seems that aprepitant is effective for the treatment of depression[74,75], and it has recently been demonstrated that it is a broad-spectrum antitumor drug[12]. Moreover, the antitumor action of the drug aprepitant against pancreatic cancer cells has been reported. In fact, aprepitant inhibits 100% of pancreatic cancer cells in a concentration-dependent manner[12].

The NK-1 receptor antagonist L-732.138 (*N*-acetyl-*L*-tryptophan 3,5-bis (trifluoromethyl) benzyl ester) (Figure 1) shows a competitive and selective antagonism for the NK-1 receptor. It is approximately 1000-fold more potent in cloned human NK-1 receptors than in cloned human NK-2 and NK-3 receptors, and approximately 200-fold more potent in human NK-1 receptors than in rat NK-1 receptors[85]. The $IC_{50}$ for the human NK-1 receptor expressed in Chinese Hamster Ovary cells is approximately 2.3 nmol[86]. It is known that the administration of L-732.138 produces an attenuation of hyperalgesia[87] and that L-732.138 is able to antagonize H(3) antagonist-induced skin vascular permeability. The antitumor action of the tryptophan-based antagonist L-732.138 against glioma, neuroblastoma and a larynx carcinoma cell lines has been also reported[80], as well as its antitumor action against pancreatic cancer cell lines[13,14].

The immunosuppressive cyclic undecapeptide cyclosporin A (CsA) is a naturally occurring fungal metabolite from *Tolypocladium inflatum Gams*. This molecule has been proposed to play a role in the treatment of human malignancies as an effective modifier of multidrug resistance. It is known that CsA has the pharmacological profile of an NK-1 receptor antagonist[88] and that CsA exerts an antitumor action due to its NK-1 receptor antagonist pharmacological profile in competition assay with SP. The antitumor action of CsA against pancreatic cancer cells occurs in a concentration-dependent manner and pancreatic tumor cells die by apoptosis[89]. However, in clinical practice this interesting therapeutic action of CsA is not possible because the high doses necessary to exert an antitumor action are associated with dangerous side effects, such as kidney failure.

Taking the above data together, it seems that the antitumor action of NK-1 receptor antagonists against pancreatic cancer cells would be due to stereochemical features and that it is not linked to the chemical composition of the antagonists[71] (Table 1), since different compounds (L-733.060, a piperidine derivative; aprepitant, a morpholine derivative; L-732.138, a tryptophane derivative ; CsA, a cyclic undecapeptide) exert an antitumor action (Figure 1). These compounds have only one thing in common: their affinity for the NK-1 receptor.

## ANTITUMOR ACTION OF THE NK-1 RECEPTOR ANTAGONISTS IS MEDIATED THROUGH THE NK-1 RECEPTOR AND TUMOR CELLS DIE BY APOPTOSIS

As reported above, the NK-1 receptor antagonists (L-733.060, L-732.138, the drug aprepitant, *etc*.) exert an antitumor action[4,5,33-38] (Figure 1). In particular, these antagonists exert this action against human glioma, larynx

carcinoma, neuroblastoma, rhabdomyosarcoma, leukemia, astrocytoma, osteosarcoma, lymphoma, retinoblastoma, melanoma, lung, breast, and gastric, and colon carcinoma cell lines[4,5,33-38,90,91], as well as against pancreatic cancer cell lines[13,14]. The antitumor action of L-733.060 against human cancer cell lines is more potent than that of aprepitant, and the antitumor action of aprepitant is more potent than that of L-732.138[4,5]. NK-1 receptor antagonists block the SP-induced mitogen stimulation of tumor cells, and they inhibit tumor cell growth in a dose-dependent manner[4,5] (Table 1).

After binding to NK-1 receptors overexpressed in tumor cells, NK-1 receptor antagonists activate the apoptotic machinery and these cells (*e.g.*, pancreatic cancer, *etc.*) die by apoptosis[4,5,12,33,34,38]. Thus, the induction of apoptosis represents a highly suitable approach to cancer treatment, although currently little is known about the mechanisms responsible for the induction of apoptosis in tumor cells. Despite this, it has been reported that the blockade of NK-1 receptors by NK-1 receptor antagonists inhibits the basal kinase activity of Akt. Tumor cells develop strategies to neutralize the multiple pathways leading to cell death, and it has been suggested that one of the most important of these is the expression of the NK-1 receptor[92]. This strategy renders tumor cells highly dependent on the SP stimulus, which provides a potent mitotic signal. This signal could counteract the different death signal pathways activated in tumor cells. The absence of the mitotic signal when the receptor is blocked with NK-1 receptor antagonists could tilt the balance within the cell to favouring apoptotic/death signals, and hence the cell would die[92]. The data reported suggest that NK-1 receptor antagonists could inhibit a large number of tumor cell types in which NK-1 receptors are overexpressed[3-5,33,34,37], and that NK-1 receptor antagonists could be candidates for broad-spectrum antineoplastic drugs including pancreatic cancer[3-5,13,14]. In general, NK-1 receptor antagonists are safe, since the administration of NK-1 receptor antagonists does not induce serious side effects[5,72,93-96], although headaches, hiccupping, vertigo and drowsiness have been reported in humans after their administration[71,95,96] (Table 1). The safety of aprepitant against human fibroblasts has been also demonstrated: the $IC_{50}$ for fibroblasts is three times higher than the $IC_{50}$ for tumor cells[12]. Moreover, the $IC_{50}$ for non-tumor cells is 90 $\mu$mol/L but the $IC_{100}$ for tumor cells is 60 $\mu$mol/L approximately[12].

Furthermore, it has been reported that the use of chemotherapy and/or radiation therapy and NK-1 receptor antagonists affords a synergistic antitumor action and decreases the side effects of chemotherapy and radiation therapy[5,97,98] (Table 1). Furthermore, it has been suggested that the co-administration of NK-1 receptor antagonists and microtubule-destabilizing agents (*e.g.*, vinblastine) could be useful in cancer, since these compounds have a synergic effect[5,98] (Table 1). This combination is synergistic for the growth inhibition of NK-1 receptor-possessing cancer cells, but not for normal cells. A better

understanding of the mechanisms underlying this interaction is needed in order to assess the clinical relevance of this novel synergistic combination. Moreover, synergism has been reported for the combination of L-733.060 with common cytostatic drugs (adriamycin, mitomycin, ifosfamide, cisplatin) in MG-63 human osteosarcoma cells, but not in non-malignant HEK293 cells[99]. Pretreatment of HEK293 with L-733.060 prior to exposure to cytostatic drugs partially protected HEK293 cells from inhibition by these drugs[99].

## NK-1 RECEPTOR ANTAGONISTS INHIBIT ANGIOGENESIS IN PANCREATIC CANCER XENOGRAFTS

Neovascularization or neoangiogenesis is a sequential process, with early endothelial proliferation followed by new vessel formation and increased blood flow, accompanied by maturation of endogenous neurovascular regulatory systems occurring late in this process in inflamed tissues[100]. The growth of new vessels from a pre-existing vasculature is a common feature of chronic inflammation (early neoangiogenesis is a key step in the transition from acute to persistent inflammation) and wound healing. Neoangiogenesis, a hallmark of tumor development, has also been associated with increased tissue innervation and the expression of NK-1 receptors. In a large majority of tumors investigated, SP and NK-1 receptors are found in the intra and peritumor blood vessels[6]. These findings have been reported specifically in pancreatic cancer[10]. SP, a main mediator of neurogenic inflammation through the release of the peptide from peripheral nerve terminals, is involved in the growth of capillary vessels *in vivo* and in the proliferation of cultured endothelial cells *in vitro*. Additionally, it is known that the proliferation of endothelial cells by NK-1 receptor agonists (SP or SP analog agonists) increases in a concentration-dependent manner (NK-1 receptor antagonists block the proliferative action of SP), whereas the action of selective NK-2 and NK-3 receptor agonists has no significant effects on the proliferation of endothelial cells. These findings indicate that NK-1 receptor agonists (*e.g.*, SP) can stimulate the process of neovascularization directly, probably through the induction of endothelial cell proliferation[101], and that SP enhanced angiogenesis results from a direct action on microvascular NK-1 receptors. Thus, through such receptors found at high density in blood vessels SP may strongly influence vascular structure and function inside and around tumors by increasing tumor blood flow and by fostering stromal development[6]. By contrast, it has been reported that NK-1 receptor antagonists inhibit endothelial cell proliferation and angiogenesis in a concentration-dependent manner[101] (Table 1). It has also been reported that the [D-Arg1, D-Trp5,7,9, Leu11] SP analog antagonist (SPA, broad-spectrum GPCR antagonist, peptide NK-1 receptor antagonist) has an antitumor action[11]. It is known

that in ductal pancreatic cancer cells expressing NK-1 receptors, NK-1 receptor antagonists induce the synthesis of proangiogenic chemokines and that in HPAF-II, a well-differentiated pancreatic cancer cell line, peptide NK-1 receptor antagonists inhibit $Ca^{2+}$ mobilization and DNA synthesis[11]. These antagonists also significantly attenuated the growth of HPAF-II tumor xenografts in nude mice beyond the treatment period. Interestingly, one peptide NK-1 receptor antagonist (SPA, broad-spectrum GPCR antagonist) markedly increases apoptosis but moderately decreases the proliferation marker Ki-67 in tumor xenografts, implying additional mechanisms for the significant growth inhibitory effect[11]. HPAF-II cells express ELR$^+$ CXC chemokines, including interleukin-8/CXCL8, which bind to CXCR2 (a member of the GPCR superfamily) and promote angiogenesis in many types of cancer, including pancreatic cancer. A salient feature of these results is that peptide NK-1 receptor antagonists markedly reduced tumor-associated angiogenesis in HPAF-II xenografts *in vivo*. The data suggest that peptide NK-1 receptor antagonists (SPA, broad-spectrum GPCR antagonist) attenuate tumor growth in pancreatic cancer *via* a dual mechanism involving both antiproliferative and antiangiogenic properties[11]. Thus, the dual-inhibitory effect of peptide NK-1 receptor antagonists could be of significant therapeutic value in pancreatic cancer, when used in combination with other anticancer drugs. In sum, all these data indicate that the SP/NK-1 receptor system controls neoangiogenesis in pancreatic cancer and that, in addition, this system could also regulate the growth of the pancreatic tumoral mass, since NK-1 receptors are overexpressed in tumoral cells and in peritumoral pancreatic cancer tissues[10]. Thus, by using NK-1 receptor antagonists (peptide or non-peptide), the NK-1 receptor could be used as a target to inhibit both neoangiogenesis and the growth of pancreatic cancer (Figure 1 and Table 1).

Accordingly, targeted therapies for pancreatic cancer offer new ways to search for potentially more effective strategies. Thus, the use of NK-1 receptor antagonists in chronic pancreatitis could: (1) improve chronic inflammation; (2) improve pain; and (3) prevent the chronic pancreatitis associated with cancer. The use of NK-1 receptor antagonists in pancreatic cancer could exert: (1) an antitumor action, by inhibiting pancreatic cancer cell proliferation (tumor cells die by apoptosis); (2) antiangiogenic properties; and (3) inhibition of the migration of pancreatic cancer cells (preventing invasion, infiltration and metastasis). Thus, the antitumor action of NK-1 receptor antagonists in pancreatic cancer could be specifically for a single target: the NK-1 receptor (Figure 1). The mechanisms of action of NK-1 receptor antagonists are the opposite of those involved in classic chemotherapy. In addition, NK-1 receptor antagonists not only exert an antitumor action, but also elicit beneficial effects in the host such as anti-inflammatory, analgesic, anxiolytic, antidepressant, antiemetic, hepatoprotector and neuroprotector effects[4,5] (Table 1).

## SAFETY OF NK-1 RECEPTOR ANTAGONISTS IN HUMAN CLINICAL TRIALS

As reported above, an upregulation of the SP/NK-1 receptor system occurs in human pancreatic cancer cells and hence the NK-1 receptor can be considered as an important target for the treatment of this disease. The overexpression of the NK-1 receptor in human pancreatic cancer cells suggests that the administration of NK-1 receptor antagonists is an excellent strategy for the treatment of this disease (these antagonists, after binding to NK-1 receptors, induce the apoptosis of tumor cells) and in addition fewer side effects should be expected after the administration of these drugs to patients, since NK-1 receptor antagonists are specific for a determined target, the NK-1 receptor, which is overexpressed in cancer cells and it is involved in the viability of tumor cells[3]. It should be noted that the IC$_{100}$ for cancer cells is 60 μmol/L approximately but the IC$_{50}$ for non-tumor cells is 90 μmol/L[12].

Many studies have reported the absence of serious side effects when non-peptide NK-1 receptor antagonists have been administered to humans[71]. It is known that the NK-1 receptor antagonist GR-205171 alleviated anxious symptoms in patients with social phobia[102]. Several non-peptide NK-1 receptor antagonists (*e.g.*, casopitant, orvepitant, vestipitant, vofopitant) have been also tested in human clinical trials for the treatment of depression, anxiety disorders, post-traumatic stress disorder, alcoholism, panic disorder and schizophrenia[103,104]. In some trials, these antagonists exerted an anxiolytic or an antidepressant action and in all the cases showed a low side effect profile. Moreover, the analgesic action of the NK-1 receptor antagonists aprepitant, lanepitant (LY-303870), AV-608 and CJ-11.974 has been tested in human trials and in all the cases the drug was ineffective in relieving pain (*e.g.*, neuropathic pain, visceral pain, osteoarthritis, fibromyalgia)[105]. However, the NK-1 receptor antagonist CP-99994 decreased postoperative dental pain[106]. NK-1 receptor antagonists have been also tested for the treatment of migraine. Thus, lanepitant was ineffective in migraine prevention and acute migraine; RPR-100.893 had no effects on migraine attacks; L-758.298 failed to abort migraine attacks, and GR-205171 was ineffective against the treatment of migraine[106]. Moreover, it has been reported that HIV-infected adults not receiving antiretroviral therapy, low (125 mg) and high (250 mg) doses of aprepitant (daily, for 14 d) were found to be safe[107]. Neurological adverse events (headache, hypersomnia, lightheadedness, dizziness) were observed in the 50% of the patients that received a higher dose of the NK-1 receptor antagonist, whereas insomnia was reported in those treated with 125 mg of aprepitant (11.1% patients). In both groups, the concentration of SP in plasma decreased. Gastrointestinal, ocular/visual, dermatological and systemic adverse events were also reported in the patients treated with aprepitant[107]. No changes in sleep

quality, anxious mood, depressed mood or neurocognitive measures were found[108].

Despite the large number of non-peptide NK-1 receptor antagonists reported, the only NK-1 receptor antagonist used currently in clinical practice is the drug aprepitant (Emend, MK-869, L-754.030) (oral) and its intravenously administered prodrug, fosaprepitant[3]. Fosaprepitant is rapidly converted to aprepitant *via* the action of ubiquitous phosphatases[108]. Both NK-1 receptor antagonists are used for the prevention of chemotherapy-induced nausea and vomiting and post-operative nausea and vomiting[70]. Many clinical human trials have reported the efficacy and safety of aprepitant/fosaprepitant for the treatment of emesis[70]. No serious adverse events were found. Aprepitant was well tolerated: no grade 3 or higher toxicities related to aprepitant were reported, whereas the adverse events mostly observed were fatigue, diarrhoea, febrile neutropenia, headache, dyspnea, constipation and hiccups[109].

Accordingly, novel possibilities for translational research are emerging for improving the treatment of diseases in which the SP/NK-1 receptor system is upregulated and hence, in particular, the use of NK-1 receptor antagonists in oncology therapy is quite promising according to the data obtained from preclinical studies[3]. Aprepitant is an excellent candidate for testing its antitumor, antimigratory and antiangiogenic action in human clinical trials since a large part of the required safety and characterization studies for aprepitant have already been carried out (aprepitant is already available in clinical practice for the treatment of emesis)[70]. Moreover, aprepitant has been developed as a nanoparticle formulation to enhance exposure and to minimize food effects. In humans, the nanoparticle formulation increased 3 times-4 times the bioavailability of this NK-1 receptor antagonist[110]. It has been also demonstrated in an *in vivo* study that fosaprepitant reduced significantly the tumor volume of MG-63 human osteosarcoma xenografts[99].

It seems that by increasing the number of days on which aprepitant is currently administered and using higher doses of aprepitant than those used in chemotherapy-induced nausea and vomiting this NK-1 receptor antagonist could be effective in cancer (*e.g.*, pancreatic cancer)[3]. However, these issues should be investigate in depth. By increasing the dose of aprepitant, higher and undescribed side effects may occur, although it has been reported that in patients with depression a dose of 300 mg/d of aprepitant was well tolerated and no significant difference in the frequency of adverse events was observed as compared with placebo[3].

## CONCLUSION

The SP/NK-1 receptor system plays an important role in the development of pancreatic cancer, neoangiogenesis and metastasis. It seems that SP acts as a mitogen for pancreatic tumor cells overexpressing NK-1 receptors and that NK-1 receptor antagonists also induce apopto-

sis in tumor cells. Research into the involvement of the SP/NK-1 receptor system in pancreatic cancer must continue in forthcoming years since it is necessary to explore new and effective therapeutic interventions in pancreatic cancer research. It is important to seek strategies targeting tumor-specific molecular derangements. This is the case of the NK-1 receptor, which is overexpressed in pancreatic tumor cells and tumor samples. NK-1 receptor antagonists induce the death of tumor cells by apoptosis. Accordingly, the NK-1 receptor is a promising target in the treatment of pancreatic cancer and NK-1 receptor antagonists could be considered as drugs for the treatment of this tumor. This conclusion is based on the following data: (1) after binding to the NK-1 receptor, SP induces pancreatic tumor cell proliferation, angiogenesis and the migration of pancreatic tumor cells (invasion, infiltration and metastasis); and (2) by contrast, NK-1 receptor antagonists inhibit pancreatic tumor cell proliferation (tumor cells die by apoptosis), have antiangiogenic properties in pancreatic cancer, and block the migratory activity of pancreatic tumor cells. Currently, in clinical practice there are few new drugs against the treatment of pancreatic cancer. However, it has been demonstrated *in vitro* and *in vivo* that NK-1 receptor antagonists exert an antitumor activity against pancreatic cancer cells. At the present, there are more than 300 NK-1 receptor antagonists[69] and this means that there are more than 300 potential drugs against the treatment of pancreatic cancer. Thus, it is crucial to test the antitumor action of NK-1 receptor antagonists in human clinical trials. In this sense, the antitumor action of NK-1 receptor antagonists already available in clinical practice for the treatment of emesis (*e.g.*, aprepitant) should be tested in clinical trials. It has previously been reported that the administration of aprepitant is well tolerated and is associated with minimal side effects. Indeed, at 300 mg/d of aprepitant was well tolerated and no significant difference in the frequency of adverse events were observed in comparison with placebo administration[71]. It is also known that, *in vitro*, aprepitant exerts an antitumor action against human pancreatic tumor cells[12]. In sum, all the data point to the notion that the NK-1 receptor could be a new and promising therapeutic target in pancreatic cancer and that NK-1 receptor antagonists could open the door to a new and promising generation of anticancer drugs against pancreatic cancer.

## REFERENCES

1    **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2013.

*CA Cancer J Clin* 2013; **63**: 11-30 [PMID: 23335087 DOI: 10.3322/caac.21166]

2   **Li D**, Xie K, Wolff R, Abbruzzese JL. Pancreatic cancer. *Lancet* 2004; **363**: 1049-1057 [PMID: 15051286 DOI: 10.1016/S0140-6736(04)15841-8]

3   **Muñoz M**, Coveñas R. Involvement of substance P and the NK-1 receptor in cancer progression. *Peptides* 2013; **48**: 1-9 [PMID: 23933301]

4   **Muñoz M**, Rosso M, Coveñas R. A new frontier in the treatment of cancer: NK-1 receptor antagonists. *Curr Med Chem* 2010; **17**: 504-516 [PMID: 20015033 DOI: 10.2174/092986710790416308]

5   **Muñoz M**, Rosso M, Coveñas R. The NK-1 receptor: a new target in cancer therapy. *Curr Drug Targets* 2011; **12**: 909-921 [PMID: 21226668 DOI: 10.2174/138945011795528796]

6   **Hennig IM**, Laissue JA, Horisberger U, Reubi JC. Substance-P receptors in human primary neoplasms: tumoral and vascular localization. *Int J Cancer* 1995; **61**: 786-792 [PMID: 7790112 DOI: 10.1002/ijc.2910610608]

7   **DeFea KA**, Vaughn ZD, O'Bryan EM, Nishijima D, Déry O, Bunnett NW. The proliferative and antiapoptotic effects of substance P are facilitated by formation of a beta-arrestin-dependent scaffolding complex. *Proc Natl Acad Sci USA* 2000; **97**: 11086-11091 [PMID: 10995467 DOI: 10.1073/pnas.190276697]

8   **Feng F**, Yang J, Tong L, Yuan S, Tian Y, Hong L, Wang W, Zhang H. Substance P immunoreactive nerve fibres are related to gastric cancer differentiation status and could promote proliferation and migration of gastric cancer cells. *Cell Biol Int* 2011; **35**: 623-629 [PMID: 21091434 DOI: 10.1042/CBI20100229]

9   **Lewis KM**, Harford-Wright E, Vink R, Nimmo AJ, Ghabriel MN. Walker 256 tumour cells increase substance P immunoreactivity locally and modify the properties of the blood-brain barrier during extravasation and brain invasion. *Clin Exp Metastasis* 2013; **30**: 1-12 [PMID: 22610781 DOI: 10.1007/s10585-012-9487-z]

10  **Friess H**, Zhu Z, Liard V, Shi X, Shrikhande SV, Wang L, Lieb K, Korc M, Palma C, Zimmermann A, Reubi JC, Büchler MW. Neurokinin-1 receptor expression and its potential effects on tumor growth in human pancreatic cancer. *Lab Invest* 2003; **83**: 731-742 [PMID: 12746482]

11  **Guha S**, Eibl G, Kisfalvi K, Fan RS, Burdick M, Reber H, Hines OJ, Strieter R, Rozengurt E. Broad-spectrum G protein-coupled receptor antagonist, [D-Arg1,D-Trp5,7,9,Leu11]SP: a dual inhibitor of growth and angiogenesis in pancreatic cancer. *Cancer Res* 2005; **65**: 2738-2745 [PMID: 15805273 DOI: 10.1158/0008-5472.CAN-04-3197]

12  **Muñoz M**, Rosso M. The NK-1 receptor antagonist aprepitant as a broad spectrum antitumor drug. *Invest New Drugs* 2010; **28**: 187-193 [PMID: 19148578 DOI: 10.1007/s10637-009-9218-8]

13  **Muñoz M**, Rosso M, Coveñas R. The NK-1 receptor is involved in the antitumoural action of L-733,060 and in the mitogenic action of substance P on human pancreatic cancer cell lines. *Lett Drug Des* Discov 2006; **3**: 323-329 [DOI: 10.2174/157018006777574168]

14  **Li X**, Ma G, Ma Q, Li W, Liu J, Han L, Duan W, Xu Q, Liu H, Wang Z, Sun Q, Wang F, Wu E. Neurotransmitter substance P mediates pancreatic cancer perineural invasion via NK-1R in cancer cells. *Mol Cancer Res* 2013; **11**: 294-302 [PMID: 23345604 DOI: 10.1158/1541-7786.MCR-12-0609]

15  **Douglas SD**, Leeman SE. Neurokinin-1 receptor: functional significance in the immune system in reference to selected infections and inflammation. *Ann N Y Acad Sci* 2011; **1217**: 83-95 [PMID: 21091716 DOI: 10.1111/j.1749-6632.2010.05826.x]

16  **Otsuka M**, Yoshioka K. Neurotransmitter functions of mammalian tachykinins. *Physiol Rev* 1993; **73**: 229-308 [PMID: 7682720]

17  **Ho WZ**, Douglas SD. Substance P and neurokinin-1 receptor

18  **Almeida TA**, Rojo J, Nieto PM, Pinto FM, Hernandez M, Martín JD, Candenas ML. Tachykinins and tachykinin receptors: structure and activity relationships. *Curr Med Chem* 2004; **11**: 2045-2081 [PMID: 15279567 DOI: 10.2174/0929867043364748]

19  **Satake H**, Kawada T. Overview of the primary structure, tissue-distribution, and functions of tachykinins and their receptors. *Curr Drug Targets* 2006; **7**: 963-974 [PMID: 16918325 DOI: 10.2174/138945006778019273]

20  **Kwatra MM**, Schwinn DA, Schreurs J, Blank JL, Kim CM, Benovic JL, Krause JE, Caron MG, Lefkowitz RJ. The substance P receptor, which couples to Gq/11, is a substrate of beta-adrenergic receptor kinase 1 and 2. *J Biol Chem* 1993; **268**: 9161-9164 [PMID: 7683643]

21  **Raddatz R**, Crankshaw CL, Snider RM, Krause JE. Similar rates of phosphatidylinositol hydrolysis following activation of wild-type and truncated rat neurokinin-1 receptors. *J Neurochem* 1995; **64**: 1183-1191 [PMID: 7532207 DOI: 10.1046/j.1471-4159.1995.64031183.x]

22  **Quartara L**, Maggi CA. The tachykinin NK1 receptor. Part I: ligands and mechanisms of cellular activation. *Neuropeptides* 1997; **31**: 537-563 [PMID: 9574822 DOI: 10.1016/S0143-4179(97)90001-9]

23  **Bowden JJ**, Garland AM, Baluk P, Lefevre P, Grady EF, Vigna SR, Bunnett NW, McDonald DM. Direct observation of substance P-induced internalization of neurokinin 1 (NK1) receptors at sites of inflammation. *Proc Natl Acad Sci USA* 1994; **91**: 8964-8968 [PMID: 7522326]

24  **Garland AM**, Grady EF, Payan DG, Vigna SR, Bunnett NW. Agonist-induced internalization of the substance P (NK1) receptor expressed in epithelial cells. *Biochem J* 1994; **303** (Pt 1): 177-186 [PMID: 7524481]

25  **Grady EF**, Garland AM, Gamp PD, Lovett M, Payan DG, Bunnett NW. Delineation of the endocytic pathway of substance P and its seven-transmembrane domain NK1 receptor. *Mol Biol Cell* 1995; **6**: 509-524 [PMID: 7545030 DOI: 10.1091/mbc.6.5.509]

26  **Mantyh PW**, Allen CJ, Ghilardi JR, Rogers SD, Mantyh CR, Liu H, Basbaum AI, Vigna SR, Maggio JE. Rapid endocytosis of a G protein-coupled receptor: substance P evoked internalization of its receptor in the rat striatum in vivo. *Proc Natl Acad Sci USA* 1995; **92**: 2622-2626 [PMID: 7535928 DOI: 10.1073/pnas.92.7.2622]

27  **Mitsuhashi M**, Ohashi Y, Shichijo S, Christian C, Sudduth-Klinger J, Harrowe G, Payan DG. Multiple intracellular signaling pathways of the neuropeptide substance P receptor. *J Neurosci Res* 1992; **32**: 437-443 [PMID: 1279191 DOI: 10.1002/jnr.490320315]

28  **Brener S**, González-Moles MA, Tostes D, Esteban F, Gil-Montoya JA, Ruiz-Avila I, Bravo M, Muñoz M. A role for the substance P/NK-1 receptor complex in cell proliferation in oral squamous cell carcinoma. *Anticancer Res* 2009; **29**: 2323-2329 [PMID: 19528498]

29  **Eistetter HR**, Mills A, Brewster R, Alouani S, Rambosson C, Kawashima E. Functional characterization of neurokinin-1 receptors on human U373MG astrocytoma cells. *Glia* 1992; **6**: 89-95 [PMID: 1328053]

30  **Esteban F**, Gonzalez-Moles MA, Castro D, Martin-Jaen Mdel M, Redondo M, Ruiz-Avila I, Muñoz M. Expression of substance P and neurokinin-1-receptor in laryngeal cancer: linking chronic inflammation to cancer promotion and progression. *Histopathology* 2009; **54**: 258-260 [PMID: 19207952 DOI: 10.1111/j.1365-2559.2008.03193.x]

31  **González Moles MA**, Mosqueda-Taylor A, Esteban F, Gil-Montoya JA, Díaz-Franco MA, Delgado M, Muñoz M. Cell proliferation associated with actions of the substance P/NK-1 receptor complex in keratocystic odontogenic tumours. *Oral Oncol* 2008; **44**: 1127-1133 [PMID: 18486533 DOI:

modulation of HIV. *J Neuroimmunol* 2004; **157**: 48-55 [PMID: 15579279]

10.1016/j.oraloncology.2008.02.010]

32    **Mukerji I**, Ramkissoon SH, Reddy KK, Rameshwar P. Autocrine proliferation of neuroblastoma cells is partly mediated through neurokinin receptors: relevance to bone marrow metastasis. *J Neurooncol* 2005; **71**: 91-98 [PMID: 15690122 DOI: 10.1007/s11060-004-9182-2]

33    **Muñoz M**, González-Ortega A, Coveñas R. The NK-1 receptor is expressed in human leukemia and is involved in the antitumor action of aprepitant and other NK-1 receptor antagonists on acute lymphoblastic leukemia cell lines. *Invest New Drugs* 2012; **30**: 529-540 [PMID: 21120581 DOI: 10.1007/s10637-010-9594-0]

34    **Muñoz M**, González-Ortega A, Rosso M, Robles-Frias MJ, Carranza A, Salinas-Martín MV, Coveñas R. The substance P/neurokinin-1 receptor system in lung cancer: focus on the antitumor action of neurokinin-1 receptor antagonists. *Peptides* 2012; **38**: 318-325 [PMID: 23026680 DOI: 10.1016/j.peptides.2012.09.024]

35    **Muñoz M**, Rosso M, Coveñas R, Montero I, González-Moles MA, Robles MJ. Neurokinin-1 receptors located in human retinoblastoma cell lines: antitumor action of its antagonist, L-732,138. *Invest Ophthalmol Vis Sci* 2007; **48**: 2775-2781 [PMID: 17525212 DOI: 10.1167/iovs.05-1591]

36    **Muñoz M**, Rosso M, Pérez A, Coveñas R, Rosso R, Zamarriego C, Piruat JI. The NK1 receptor is involved in the antitumoural action of L-733,060 and in the mitogenic action of substance P on neuroblastoma and glioma cell lines. *Neuropeptides* 2005; **39**: 427-432 [PMID: 15939468 DOI: 10.1016/j.npep.2005.03.004]

37    **Muñoz M**, Rosso M, Robles-Frias MJ, Salinas-Martín MV, Rosso R, González-Ortega A, Coveñas R. The NK-1 receptor is expressed in human melanoma and is involved in the antitumor action of the NK-1 receptor antagonist aprepitant on melanoma cell lines. *Lab Invest* 2010; **90**: 1259-1269 [PMID: 20458280 DOI: 10.1038/labinvest.2010.92]

38    **Rosso M**, Robles-Frías MJ, Coveñas R, Salinas-Martín MV, Muñoz M. The NK-1 receptor is expressed in human primary gastric and colon adenocarcinomas and is involved in the antitumor action of L-733,060 and the mitogenic action of substance P on human gastrointestinal cancer cell lines. *Tumour Biol* 2008; **29**: 245-254 [PMID: 18781096 DOI: 10.1159/000152942]

39    **Patel HJ**, Ramkissoon SH, Patel PS, Rameshwar P. Transformation of breast cells by truncated neurokinin-1 receptor is secondary to activation by preprotachykinin-A peptides. *Proc Natl Acad Sci USA* 2005; **102**: 17436-17441 [PMID: 16291810 DOI: 10.1073/pnas.0506351102]

40    **Moharita A**, Harrison, JS, Rameshwar P. Neurokinin receptors and subtypes as potential targets in breast cancer: relevance to bone marrow metastasis. *Drug Res Rev* 2004; **1**: 1-6 [DOI: 10.2174/1567269043390771]

41    **Ramkissoon SH**, Patel PS, Taborga M, Rameshwar P. Nuclear factor-kappaB is central to the expression of truncated neurokinin-1 receptor in breast cancer: implication for breast cancer cell quiescence within bone marrow stroma. *Cancer Res* 2007; **67**: 1653-1659 [PMID: 17308106 DOI: 10.1158/0008-5472.CAN-06-3813]

42    **Gillespie E**, Leeman SE, Watts LA, Coukos JA, O'Brien MJ, Cerda SR, Farraye FA, Stucchi AF, Becker JM. Truncated neurokinin-1 receptor is increased in colonic epithelial cells from patients with colitis-associated cancer. *Proc Natl Acad Sci USA* 2011; **108**: 17420-17425 [PMID: 21969570 DOI: 10.1073/pnas.1114275108]

43    **Ehlers RA**, Kim Sh, Zhang Y, Ethridge RT, Murrilo C, Hellmich MR, Evans DB, Townsend CM, Mark Evers B. Gut peptide receptor expression in human pancreatic cancers. *Ann Surg* 2000; **231**: 838-848 [PMID: 10816627]

44    **Schulz S**, Stumm R, Röcken C, Mawrin C, Schulz S. Immunolocalization of full-length NK1 tachykinin receptors in human tumors. *J Histochem Cytochem* 2006; **54**: 1015-1020 [PMID: 16651388 DOI: 10.1369/jhc.6A6966.2006]

45    **Luo W**, Sharif TR, Sharif M. Substance P-induced mitogenesis in human astrocytoma cells correlates with activation of the mitogen-activated protein kinase signaling pathway. *Cancer Res* 1996; **56**: 4983-4991 [PMID: 8895754]

46    **Lowenfels AB**, Maisonneuve P, Cavallini G, Ammann RW, Lankisch PG, Andersen JR, Dimagno EP, Andrén-Sandberg A, Domellöf L. Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group. *N Engl J Med* 1993; **328**: 1433-1437 [PMID: 8479461 DOI: 10.1056/NEJM199305203282001]

47    **Shrikhande SV**, Friess H, di Mola FF, Tempia-Caliera A, Conejo Garcia JR, Zhu Z, Zimmermann A, Büchler MW. NK-1 receptor gene expression is related to pain in chronic pancreatitis. *Pain* 2001; **91**: 209-217 [PMID: 11275376 DOI: 10.1016/S0304-3959(00)00436-X]

48    **Palma C**, Nardelli F, Manzini S, Maggi CA. Substance P activates responses correlated with tumour growth in human glioma cell lines bearing tachykinin NK1 receptors. *Br J Cancer* 1999; **79**: 236-243 [PMID: 9888463 DOI: 10.1038/sj.bjc.6690039]

49    **Nakajima Y**, Tsuchida K, Negishi M, Ito S, Nakanishi S. Direct linkage of three tachykinin receptors to stimulation of both phosphatidylinositol hydrolysis and cyclic AMP cascades in transfected Chinese hamster ovary cells. *J Biol Chem* 1992; **267**: 2437-2442 [PMID: 1370820]

50    **Takeda Y**, Blount P, Sachais BS, Hershey AD, Raddatz R, Krause JE. Ligand binding kinetics of substance P and neurokinin A receptors stably expressed in Chinese hamster ovary cells and evidence for differential stimulation of inositol 1,4,5-trisphosphate and cyclic AMP second messenger responses. *J Neurochem* 1992; **59**: 740-745 [PMID: 1321234 DOI: 10.1111/j.1471-4159.1992.tb09430.x]

51    **Akazawa T**, Kwatra SG, Goldsmith LE, Richardson MD, Cox EA, Sampson JH, Kwatra MM. A constitutively active form of neurokinin 1 receptor and neurokinin 1 receptor-mediated apoptosis in glioblastomas. *J Neurochem* 2009; **109**: 1079-1086 [PMID: 19519779 DOI: 10.1111/j.1471-4159.2009.06032.x]

52    **Fowler CJ**, Brännström G. Substance P enhances forskolin-stimulated cyclic AMP production in human UC11MG astrocytoma cells. *Methods Find Exp Clin Pharmacol* 1994; **16**: 21-28 [PMID: 7513037]

53    **Gitter BD**, Regoli D, Howbert JJ, Glasebrook AL, Waters DC. Interleukin-6 secretion from human astrocytoma cells induced by substance P. *J Neuroimmunol* 1994; **51**: 101-108 [PMID: 7512575 DOI: 10.1016/S0165-5728(97)00167-7]

54    **Johnson CL**, Johnson CG. Substance P regulation of glutamate and cystine transport in human astrocytoma cells. *Receptors Channels* 1993; **1**: 53-59 [PMID: 7521733]

55    **Medrano S**, Gruenstein E, Dimlich RV. Substance P receptors on human astrocytoma cells are linked to glycogen breakdown. *Neurosci Lett* 1994; **167**: 14-18 [PMID: 7513838 DOI: 10.1016/0304-3940(94)91017-0]

56    **Tung WL**, Lee CM. Effects of tachykinins on [3H]taurine release from human astrocytoma cells (U-373 MG). *Brain Res* 1991; **549**: 171-173 [PMID: 1716506 DOI: 10.1002/glia.440110309]

57    **Warburg O**. On the origin of cancer cells. *Science* 1956; **123**: 309-314 [PMID: 13298683 DOI: 10.1126/science.123.3191.309]

58    **Sporn MB**. The war on cancer. *Lancet* 1996; **347**: 1377-1381 [PMID: 8637346 DOI: 10.1016/S0140-6736(96)91015-6]

59    **Lang K**, Drell TL, Lindecke A, Niggemann B, Kaltschmidt C, Zaenker KS, Entschladen F. Induction of a metastatogenic tumor cell type by neurotransmitters and its pharmacological inhibition by established drugs. *Int J Cancer* 2004; **112**: 231-238 [PMID: 15352035 DOI: 10.1002/ijc.20410]

60    **Fackler OT**, Grosse R. Cell motility through plasma membrane blebbing. *J Cell Biol* 2008; **181**: 879-884 [PMID: 18541702 DOI: 10.1083/jcb.200802081]

61    **Meshki J**, Douglas SD, Lai JP, Schwartz L, Kilpatrick LE, Tuluc F. Neurokinin 1 receptor mediates membrane blebbing in

HEK293 cells through a Rho/Rho-associated coiled-coil kinase-dependent mechanism. *J Biol Chem* 2009; **284**: 9280-9289 [PMID: 19179340 DOI: 10.1074/jbc.M808825200]

62    **Reeve JG**, Bleehen NM. [D-Arg1, D-Phe5, D-Trp7,9, Leu11] substance P induces apoptosis in lung cancer cell lines in vitro. *Biochem Biophys Res Commun* 1994; **199**: 1313-1319 [PMID: 7511895 DOI: 10.1006/bbrc.1994.1374]

63    **Woll PJ**, Rozengurt E. [D-Arg1,D-Phe5,D-Trp7,9,Leu11]substance P, a potent bombesin antagonist in murine Swiss 3T3 cells, inhibits the growth of human small cell lung cancer cells in vitro. *Proc Natl Acad Sci USA* 1988; **85**: 1859-1863 [PMID: 2450349]

64    **Seckl MJ**, Higgins T, Rozengurt E. [D-Arg1,D-Trp5,7,9,Leu11]Substance P coordinately and reversibly inhibits bombesin- and vasopressin-induced signal transduction pathways in Swiss 3T3 cells. *J Biol Chem* 1996; **271**: 29453-29460 [PMID: 8910612 DOI: 10.1074/jbc.271.46.29453]

65    **Seckl MJ**, Higgins T, Widmer F, Rozengurt E. [D-Arg1,D-Trp5,7,9,Leu11]substance P: a novel potent inhibitor of signal transduction and growth in vitro and in vivo in small cell lung cancer cells. *Cancer Res* 1997; **57**: 51-54 [PMID: 8988040]

66    **Snider RM**, Constantine JW, Lowe JA, Longo KP, Lebel WS, Woody HA, Drozda SE, Desai MC, Vinick FJ, Spencer RW. A potent nonpeptide antagonist of the substance P (NK1) receptor. *Science* 1991; **251**: 435-437 [PMID: 1703323 DOI: 10.1126/science.1703323]

67    **Maggi CA**, Patacchini R, Rovero P, Giachetti A. Tachykinin receptors and tachykinin receptor antagonists. *J Auton Pharmacol* 1993; **13**: 23-93 [PMID: 8382703]

68    **Regoli D**, Boudon A, Fauchére JL. Receptors and antagonists for substance P and related peptides. *Pharmacol Rev* 1994; **46**: 551-599 [PMID: 7534932]

69    **Giardina GA**, Gagliardi S, Martinelli M. Antagonists at the neurokinin receptors--recent patent literature. *IDrugs* 2003; **6**: 758-772 [PMID: 12917772]

70    **Muñoz M**, Coveñas R. Safety of neurokinin-1 receptor antagonists. *Expert Opin Drug Saf* 2013; **12**: 673-685 [PMID: 23706125 DOI: 10.1517/14740338.2013.804059]

71    **Munoz M**, Covenas R. NK-1 receptor antagonists: a new generation of anticancer drugs. *Mini Rev Med Chem* 2012; **12**: 593-599 [PMID: 22512565 DOI: 10.2174/138955712800626692]

72    **Harrison T**, Williams BJ, Swain CJ, Ball RG. Piperidine-ether based hNK1 antagonists 1: determination of the relative and absolute stereochemical requirements. *Biomed Chem Lett* 1994: **4**: 2545-2550 [DOI: 10.1016/S0960-894X(01)80280-8]

73    **Rupniak NM**, Carlson E, Boyce S, Webb JK, Hill RG. Enantioselective inhibition of the formalin paw late phase by the NK1 receptor antagonist L-733,060 in gerbils. *Pain* 1996; **67**: 189-195 [PMID: 8895247]

74    **Kramer MS**, Cutler N, Feighner J, Shrivastava R, Carman J, Sramek JJ, Reines SA, Liu G, Snavely D, Wyatt-Knowles E, Hale JJ, Mills SG, MacCoss M, Swain CJ, Harrison T, Hill RG, Hefti F, Scolnick EM, Cascieri MA, Chicchi GG, Sadowski S, Williams AR, Hewson L, Smith D, Carlson EJ, Hargreaves RJ, Rupniak NM. Distinct mechanism for antidepressant activity by blockade of central substance P receptors. *Science* 1998; **281**: 1640-1645 [PMID: 9733503 DOI: 10.1126/science.281.5383.1640]

75    **Varty GB**, Cohen-Williams ME, Hunter JC. The antidepressant-like effects of neurokinin NK1 receptor antagonists in a gerbil tail suspension test. *Behav Pharmacol* 2003; **14**: 87-95 [PMID: 12576885]

76    **Rupniak NM**, Carlson EC, Harrison T, Oates B, Seward E, Owen S, de Felipe C, Hunt S, Wheeldon A. Pharmacological blockade or genetic deletion of substance P (NK(1)) receptors attenuates neonatal vocalisation in guinea-pigs and mice. *Neuropharmacology* 2000; **39**: 1413-1421 [PMID: 10818257 DOI: 10.1016/S0028-3908(00)00052-6]

77    **Bang R**, Sass G, Kiemer AK, Vollmar AM, Neuhuber WL, Tiegs G. Neurokinin-1 receptor antagonists CP-96,345 and L-733,060 protect mice from cytokine-mediated liver injury.

*J Pharmacol Exp Ther* 2003; **305**: 31-39 [PMID: 12649350 DOI: 10.1124/jpet.102.043539]

78    **Muñoz M**, Pérez A, Rosso M, Zamarriego C, Rosso R. Antitumoral action of the neurokinin-1 receptor antagonist L-733 060 on human melanoma cell lines. *Melanoma Res* 2004; **14**: 183-188 [PMID: 15179186]

79    **Muñoz M**, Rosso M, Pérez A, Coveñas R, Rosso R, Zamarriego C, Soult JA, Montero I. Antitumoral action of the neurokinin-1-receptor antagonist L-733,060 and mitogenic action of substance P on human retinoblastoma cell lines. *Invest Ophthalmol Vis Sci* 2005; **46**: 2567-2570 [PMID: 15980249 DOI: 10.1167/iovs.04-1530]

80    **Muñoz M**, Rosso M, Aguilar FJ, González-Moles MA, Redondo M, Esteban F. NK-1 receptor antagonists induce apoptosis and counteract substance P-related mitogenesis in human laryngeal cancer cell line HEp-2. *Invest New Drugs* 2008; **26**: 111-118 [PMID: 17906845 DOI: 10.1007/s10637-007-9087-y]

81    **Muñoz M**, Pérez A, Coveñas R, Rosso M, Castro E. Antitumoural action of L-733,060 on neuroblastoma and glioma cell lines. *Arch Ital Biol* 2004; **142**: 105-112 [PMID: 15248566]

82    **Humphrey JM**. Medicinal chemistry of selective neurokinin-1 antagonists. *Curr Top Med Chem* 2003; **3**: 1423-1435 [PMID: 12871173]

83    **Tattersall FD**, Rycroft W, Cumberbatch M, Mason G, Tye S, Williamson DJ, Hale JJ, Mills SG, Finke PE, MacCoss M, Sadowski S, Ber E, Cascieri M, Hill RG, MacIntyre DE, Hargreaves RJ. The novel NK1 receptor antagonist MK-0869 (L-754,030) and its water soluble phosphoryl prodrug, L-758,298, inhibit acute and delayed cisplatin-induced emesis in ferrets. *Neuropharmacology* 2000; **39**: 652-663 [PMID: 10728886 DOI: 10.1016/S0028-3908(99)00172-0]

84    **Navari RM**. Fosaprepitant (MK-0517): a neurokinin-1 receptor antagonist for the prevention of chemotherapy-induced nausea and vomiting. *Expert Opin Investig Drugs* 2007; **16**: 1977-1985 [PMID: 18042005 DOI: 10.1517/13543784.16.12.1977]

85    **MacLeod AM**, Merchant KJ, Brookfield F, Kelleher F, Stevenson G, Owens AP, Swain CJ, Casiceri MA, Sadowski S, Ber E. Identification of L-tryptophan derivatives with potent and selective antagonist activity at the NK1 receptor. *J Med Chem* 1994; **37**: 1269-1274 [PMID: 7513763 DOI: 10.1021/jm00035a006]

86    **Cascieri MA**, Macleod AM, Underwood D, Shiao LL, Ber E, Sadowski S, Yu H, Merchant KJ, Swain CJ, Strader CD. Characterization of the interaction of N-acyl-L-tryptophan benzyl ester neurokinin antagonists with the human neurokinin-1 receptor. *J Biol Chem* 1994; **269**: 6587-6591 [PMID: 7509807]

87    **Cahill CM**, Coderre TJ. Attenuation of hyperalgesia in a rat model of neuropathic pain after intrathecal pre- or post-treatment with a neurokinin-1 antagonist. *Pain* 2002; **95**: 277-285 [PMID: 11839427 DOI: 10.1016/S0304-3959(01)00410-9]

88    **Gitter BD**, Waters DC, Threlkeld PG, Lovelace AM, Matsumoto K, Bruns RF. Cyclosporin A is a substance P (tachykinin NK1) receptor antagonist. *Eur J Pharmacol* 1995; **289**: 439-446 [PMID: 7556412 DOI: 10.1016/0922-4106(95)90152-3]

89    **Muñoz M**, Rosso M, González A, Saenz J, Coveñas R. The broad-spectrum antitumor action of cyclosporin A is due to its tachykinin receptor antagonist pharmacological profile. *Peptides* 2010; **31**: 1643-1648 [PMID: 20542069 DOI: 10.1016/j.peptides.2010.06.002]

90    **Bigioni M**, Benzo A, Irrissuto C, Maggi CA, Goso C. Role of NK-1 and NK-2 tachykinin receptor antagonism on the growth of human breast carcinoma cell line MDA-MB-231. *Anticancer Drugs* 2005; **16**: 1083-1089 [PMID: 16222150]

91    **Huang WQ**, Wang JG, Chen L, Wei HJ, Chen H. SR140333 counteracts NK-1 mediated cell proliferation in human breast cancer cell line T47D. *J Exp Clin Cancer Res* 2010; **29**: 55 [PMID: 20497542 DOI: 10.1186/1756-9966-29-55]

92    **Esteban F**, Muñoz M, González-Moles MA, Rosso M. A role for substance P in cancer promotion and progression:

a mechanism to counteract intracellular death signals following oncogene activation or DNA damage. *Cancer Metastasis Rev* 2006; **25**: 137-145 [PMID: 16680578 DOI: 10.1007/s10555-006-8161-9]

93    **Choi MR**, Jiles C, Seibel NL. Aprepitant use in children, adolescents, and young adults for the control of chemotherapy-induced nausea and vomiting (CINV). *J Pediatr Hematol Oncol* 2010; **32**: e268-e271 [PMID: 20736848 DOI: 10.1097/MPH.0b013e3181e5e1af]

94    **Paul B**, Trovato JA, Thompson J, Badros AZ, Goloubeva O. Efficacy of aprepitant in patients receiving high-dose chemotherapy with hematopoietic stem cell support. *J Oncol Pharm Pract* 2010; **16**: 45-51 [PMID: 19525301 DOI: 10.1177/1078155209105399]

95    **Roila F**, Rolski J, Ramlau R, Dediu M, Russo MW, Bandekar RR, Grunberg SM. Randomized, double-blind, dose-ranging trial of the oral neurokinin-1 receptor antagonist casopitant mesylate for the prevention of cisplatin-induced nausea and vomiting. *Ann Oncol* 2009; **20**: 1867-1873 [PMID: 19541792 DOI: 10.1093/annonc/mdp194]

96    **Ständer S**, Siepmann D, Herrgott I, Sunderkötter C, Luger TA. Targeting the neurokinin receptor 1 with aprepitant: a novel antipruritic strategy. *PLoS One* 2010; **5**: e10968 [PMID: 20532044 DOI: 10.1371/journal.pone.0010968]

97    **Kitchens CA**, McDonald PR, Pollack IF, Wipf P, Lazo JS. Synergy between microtubule destabilizing agents and neurokinin 1 receptor antagonists identified by an siRNA synthetic lethal screen. *FASEB J* 2009; **23**: 756

98    **Alfieri AB**, Cubeddu LX. Efectos de los antagonistas de los receptores NK1 y de la dexametasona sobre la inflamación neurogénica inducida por ciclofosfamida y por radiación X, en la rata. *AVFT* 2004; **23**: 61-66

99    **Muñoz M**, Berger M, Rosso M, Gonzalez-Ortega A, Carranza A, Coveñas R. Antitumor activity of neurokinin-1 receptor antagonists in MG-63 human osteosarcoma xenografts. *Int J Oncol* 2014; **44**: 137-146 [PMID: 24190675 DOI: 10.3892/ijo.2013.2164]

100   **Walsh DT**, Weg VB, Williams TJ, Nourshargh S. Substance P-induced inflammatory responses in guinea-pig skin: the effect of specific NK1 receptor antagonists and the role of endogenous mediators. *Br J Pharmacol* 1995; **114**: 1343-1350 [PMID: 7541689]

101   **Ziche M**, Morbidelli L, Pacini M, Geppetti P, Alessandri G, Maggi CA. Substance P stimulates neovascularization in vivo and proliferation of cultured endothelial cells. *Microvasc Res* 1990; **40**: 264-278 [PMID: 1701206]

102   **Furmark T**, Appel L, Michelgård A, Wahlstedt K, Ahs F, Zancan S, Jacobsson E, Flyckt K, Grohp M, Bergström M, Pich EM, Nilsson LG, Bani M, Långström B, Fredrikson M. Cerebral blood flow changes after treatment of social phobia with the neurokinin-1 antagonist GR205171, citalopram, or placebo. *Biol Psychiatry* 2005; **58**: 132-142 [PMID: 16038684 DOI: 10.1016/j.biopsych.2005.03.029]

103   **Ebner K**, Sartori SB, Singewald N. Tachykinin receptors as therapeutic targets in stress-related disorders. *Curr Pharm Des* 2009; **15**: 1647-1674 [PMID: 19442179 DOI: 10.2174/138161209788168074]

104   **Gilman JM**, Hommer DW. Modulation of brain response to emotional images by alcohol cues in alcohol-dependent patients. *Addict Biol* 2008; **13**: 423-434 [PMID: 18507736]

105   **Borsook D**, Upadhyay J, Klimas M, Schwarz AJ, Coimbra A, Baumgartner R, George E, Potter WZ, Large T, Bleakman D, Evelhoch J, Iyengar S, Becerra L, Hargreaves RJ. Decision-making using fMRI in clinical drug development: revisiting NK-1 receptor antagonists for pain. *Drug Discov Today* 2012; **17**: 964-973 [PMID: 22579743 DOI: 10.1016/j.drudis.2012.05.004]

106   **Dionne RA**, Max MB, Gordon SM, Parada S, Sang C, Gracely RH, Sethna NF, MacLean DB. The substance P receptor antagonist CP-99,994 reduces acute postoperative pain. *Clin Pharmacol Ther* 1998; **64**: 562-568 [PMID: 9834049 DOI: 10.1016/S0009-9236(98)90140-0]

107   **Tebas P**, Tuluc F, Barrett JS, Wagner W, Kim D, Zhao H, Gonin R, Korelitz J, Douglas SD. A randomized, placebo controlled, double masked phase IB study evaluating the safety and antiviral activity of aprepitant, a neurokinin-1 receptor antagonist in HIV-1 infected adults. *PLoS One* 2011; **6**: e24180 [PMID: 21931661 DOI: 10.1371/journal.pone.0024180]

108   **Saito H**, Yoshizawa H, Yoshimori K, Katakami N, Katsumata N, Kawahara M, Eguchi K. Efficacy and safety of single-dose fosaprepitant in the prevention of chemotherapy-induced nausea and vomiting in patients receiving high-dose cisplatin: a multicentre, randomised, double-blind, placebo-controlled phase 3 trial. *Ann Oncol* 2013; **24**: 1067-1073 [PMID: 23117073 DOI: 10.1093/annonc/mds541]

109   **Abidi MH**, Tageja N, Ayash L, Abrams J, Ratanatharathorn V, Al-Kadhimi Z, Lum L, Cronin S, Ventimiglia M, Uberti J. Aprepitant for prevention of nausea and vomiting secondary to high-dose cyclophosphamide administered to patients undergoing autologous peripheral blood stem cells mobilization: a phase II trial. *Support Care Cancer* 2012; **20**: 2363-2369 [PMID: 22193771 DOI: 10.1007/s00520-011-1341-3]

110   **Olver I**, Shelukar S, Thompson KC. Nanomedicines in the treatment of emesis during chemotherapy: focus on aprepitant. *Int J Nanomedicine* 2007; **2**: 13-18 [PMID: 17722507]

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