ANSWERING REVIEWERS

December 23, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6891-review.doc).

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

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Name of Journal: *World Journal of Gastroenterology*

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The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer
(1) Not indicate any comment
(2) See below
(3) Not indicate any comment

REFEREE 2

Points 1-4: The referee said: *1.) The authors indicate that drugs with specificity for pancreatic cancer are urgently needed, yet the primary pathway being evaluated is active in numerous locations around the body, which would seem to be not specific to pancreatic cancer. 2.) Table one indicates that there is specific activity against pancreatic cancer cells. Yet the authors indicate that substance P is expressed throughout the body. This would suggest that drugs targeting this pathway would not in fact be specific for pancreatic cancer cells. 3.) Not discussed in any detail are the side effects associated with anti-Substance P/NK-1 therapy. These side effects will be the primary limiting factors in establishing any therapeutic benefit from this class of agents. 4.) Have there been any studies in humans with any of these agents?*

According to the suggestions of the reviewer, we have included a new section entitled "Safety of NK-1 receptor antagonists in human clinical trials". In this section, we focus the discussion on the overexpression of the NK-1 receptor in human pancreatic cancer cells and on the possible use of NK-1 receptor antagonists for the treatment of cancer patients, since these antagonists would be quite specific against the tumor cells overexpressing the NK-1

receptor and hence fewer side effects would be expected. This latter idea is in agreement with the data reported in human clinical trials on the safety of non-peptide NK-1 receptor antagonists when they were tested in diseases in which the SP/NK-1 receptor system is altered. Thus, in the new section, we have indicated that many human clinical trials have shown that NK-1 receptor antagonists are in general safe and that they showed a low side effect profile. New references (103-111) have been also added. We think that the key-point is the upregulation of the SP/NK-1 receptor system in many diseases and, in the case of the pancreatic cancer, the overexpression of the NK-1 receptor in tumor cells.

The new section included is the following:

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 μM approximately but the IC_{50} for non-tumor cells is 90 μM [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-205,171 alleviated anxious symptoms in patients with social phobia[103]. 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 [104, 105]. 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-303,870), 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)[106]. However, the NK-1 receptor antagonist CP-99,994 decreased postoperative dental pain[107]. 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-205,171 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 days) were found to be safe [108]. 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 [108]. 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[109]. Both NK-1 receptor antagonists are used for the prevention of chemotherapy-induced nausea and vomiting and post-operative nausea and vomiting[71]. Many clinical human trials have reported the efficacy and safety of aprepitant/fosaprepitant for the treatment of emesis[71]. 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 nausea and vomiting, fatigue, diarrhoea, febrile neutropenia, headache, dyspnea, constipation and hiccups[110].

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)[71]. Moreover, aprepitant has been developed as a nanoparticle formulation to enhance exposure and to minimize food effects[111]. In humans, the nanoparticle formulation increased 3-4 times the bioavailability of this NK-1 receptor antagonist[111]. It has been also demonstrated in an *in vivo* study that fosaprepitant reduced significantly the tumor volume of MG-63 human osteosarcoma xenografts[100].

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/day of aprepitant was well tolerated and no significant difference in the frequency of adverse events was observed as compared with placebo[3].

Point 5: The referee said: *Though in theory agents with specificity for this pathway might seem to be "intelligent bullets", it remains entirely unproven whether the ratio of efficacy to toxicity will allow the use of thes agents in clinical practice.*

The sentence in which this term was included has been deleted in the new version.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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