SCIENTIFIC DISCUSSION

1. Introduction

Aloxi contains palonosetron, a 5-hydroxytryptamine (serotonin) type 3 receptor antagonist, as active substance. With the present application, the applicant sought a marketing authorisation in the following indication:

- "the prevention of acute nausea and vomiting associated with initial and repeat course of moderately and highly emetogenic cancer chemotherapy and
- the prevention of delayed nausea and vomiting associated with initial and repeat courses of moderately emetogenic chemotherapy."

Chemotherapy-induced nausea and vomiting (CINV)

CINV can be broadly categorised as acute, when nausea and vomiting occur within 24 hours after the start of chemotherapy [1], delayed, if CINV persist for 6 to 7 days after therapy [2, 3], or anticipatory, if CINV occur prior to chemotherapy administration [2, 4]. Chemotherapy agents have a highly variable emetogenic potential and can be classified according to their emetogenic level as agents with low, intermediate and high emetogenic risk. CINV remains a significant side effect experienced by cancer patients especially when treated with highly emetogenic regimens. It can impair patient's quality of life and in case it becomes serious, dehydration, malnutrition, metabolic disturbances, and aspiration pneumonia may occur. As a consequence, control of nausea and vomiting plays an important part in the overall treatment success for cancer patients.

The precise mechanisms by which chemotherapy induces nausea and vomiting are unknown. However, it appears probable that different chemotherapeutic agents act at different sites and that some chemotherapeutic agents act at multiple sites [5]. The mechanisms by which chemotherapeutic agents cause nausea and vomiting are activation of the chemoreceptor trigger zone (CTZ) either directly or indirectly, peripheral stimulation of the gastrointestinal tract, vestibular mechanisms, cortical mechanisms, or alterations of taste and smell [6]. For the majority of the chemotherapeutic agents, the most common mechanism is thought to be activation of the CTZ. The CTZ is located in the area postrema of the brain and can be reached via the cerebrospinal fluid or the blood. Some of the neurotransmitters located in the area postrema of the brain that may be exited and lead to emesis include dopamine, serotonine, histamine, norepinephrine, apomorphine, neurotensin, angiotensin II, vasoactive intestinal polypeptide, gastrin, vasopressin, thyrotopin-releasing hormone, leucine enkephalin and substance P [7].

5-HT_3 receptor antagonists

It appears that 5-hydroxytryptamine (5-HT) receptors are particularly important in the pathophysiology of acute vomiting [6]. Delayed emesis is observed in as many as 80 % of patients, typically occurring 24 to 72 hours after high total doses of cisplatin (>100 mg/m^2) have been administered. To date, the pathophysiology of delayed emesis remains unclear [8]. The precise mechanism of action of the 5-HT-receptor antagonists is unknown; however, they may have both a central and a peripheral effect. The gastrointestinal tract contains 80 % of the body's supply of serotonin. During chemotherapy, enterochromaffin cells that line the gastrointestinal tract are damaged resulting in the release of serotonin. Serotonin stimulates vagal afferent neurons that activates the vomiting center or directly activate the chemotherapy trigger zone.

A multitude of 5-HT-receptor subtypes have been cloned to date [9], which are expressed in distinct but often overlapping patterns [10]. Four 5-HT-receptors families with defined functions, 5-HT_1 through 5-HT_4, currently are recognized. The 5-HT_{1-2} and 5-HT_{4-7} receptor families are members of the superfamiliy of G protein-coupled receptors with a predicted membrane topology composed of an extracellular *N*-terminal segment linked to an intracellular C terminus by seven transmembrane-spanning segments. The 5-HT_3 receptor, on the other hand, is a ligand-gated ion channel that gates

Na+ and K+ and has a predicted membrane topology akin to that of the nicotinic cholinergic receptor [9].
5-HT_3 inhibitors block serotonin receptors and subsequently the neuronal cascade of events leading to nausea and vomiting is in effect blunted or blocked from further activation. Studies have shown that the 5-HT_3 receptor antagonists decrease emesis from several chemotherapeutic agents, including cisplatin, cyclophosphamide and doxorubicin [11-14].

The goal of antiemetic therapy is to prevent nausea and vomiting completely. Metoclopramide, blocks emesis via the 5-HT_3 pathway at higher doses [15]. However, metoclopramide is not selective for the 5-HT_3 pathway, and development of highly selective antagonists of the 5-HT_3 receptor allowed for good antiemetic effect with a lower side effect profile [6]. In addition to 5-HT_3 receptor antagonists, substances used in the treatment of CINV include mainly steroids and dopamine D2 antagonists. Recently, a new antiemetic agent, aprepitant (an antagonist of the neurokinin 1 (NK1) receptors), has been centrally authorised for the prevention of acute and delayed nausea and vomiting associated with initial and repeated courses of highly emetogenic cancer chemotherapy, including high-dose cisplatin, in combination with other antiemetic agents.

5-HT_3 receptor antagonists used as antiemetic therapies available in some European countires include ondansetron, granisetron, tropisetron and dolasetron. Since their introduction, these agents have become the standard antiemetic agents for control of CINV and continue to provide effective management of nausea and vomiting with rarely requiring discontinuation of therapy. They are not superior to metoclopramide and are therefore not approved for use in the delayed phase by many authorities.

Palonosetron hydrochloride

Palonosetron hydrochloride (palonosetron, also referred to during the development as 08-PALO or RS-25259-197, Onicit, which has been the brand name used in the EU pre-authorisation until 13 December 2004, or Aloxi, which is the official brand name in the EU and in the US), is a novel and selective serotonin receptor subtype 3 (5-HT_3) antagonist. It has little or no affinity for other bioreceptors, including other serotonergic receptors (5-HT_1, 5-HT_2 and 5-HT_4).
Syntex Laboratories - USA was the original innovator and carried out the studies corresponding to Phase I and II. Syntex licensed palonosetron to Helsinn and completed the development of the product carrying out the Phase III studies and Phase I in special populations.
This application concerns a complete application concerning a new active substance according to article 8.3 (i) of Directive 2001/83/EC.

2. Quality aspects

Introduction

Aloxi is presented as a solution for injection containing 250 micrograms of palonosetron (as hydrochloride).

The other ingredients include mannitol, disodium edetate, sodium citrate, citric acid monohydrate, water for injections, hydrochloric acid solution and sodium hydroxide solution.

Aloxi is packed in type I clear glass vial closed with chlorobutyl siliconised rubber stopper and an aluminium flip-off seal.

2.1 Active substance

Palonosetron is a new 5-hydroxytryptamine type 3 receptor antagonist. Detailed information on quality/control of materials used in the synthesis, as well as on the synthesis itself, has been provided by the way of an active substance master file (ASMF or “EDMF”).

Palonosetron is a white to off-white crystalline powder, which is freely soluble in water. It contains 2 chiral centres, but it is synthesised solely as the (S,S) enantiomer. No racemisation has been observed during the synthesis process or under stress conditions. Two crystalline forms (I and II) and an amorphous form have been identified. The crystalline form I is produced by the commercial synthesis process. Solubility studies with form II have demonstrated that in aqueous medium it readily converts into polymorph form I, which is freely soluble in water.
Stress stability studies have shown that the main degradation pathway for palonosetron in aqueous solution is oxidation, which increases with the active substance concentration. In solid-state, palonosetron is not hygroscopic at or below 82% R.H.

- Manufacture

Palonosetron is synthesised using a five-step process, three involve chemical reactions and two correspond to purification/recrystallisation. The starting materials are commercially available. The commercial synthesis process results of optimisation of an earlier development process. Both have been used to produce batches used in non-clinical/clinical studies.
Satisfactory specifications and associated methods have been provided for the starting materials, key intermediates, reagents and solvents in the ASMF Restricted part.

- Specification

The active substance specification includes tests for appearance, identity (IR and UV), assay (reverse phase HPLC achiral), chloride content, optical rotation (PhEur), impurities (chiral HPLC), residual solvents (GC), heavy metals (PhEur), sulphated ash (PhEur), bioburden (PhEur), mould and yeast count (PhEur), bacterial endotoxins (PhEur), loss on drying (PhEur), pH of solution (PhEur), and clarity of solution (PhEur).
The formation of the desired enantiomer of palonosetron is ensured by controlling enantiomeric purity of the starting materials and of the intermediates. In addition, an optical rotation test is part of the specification and a chiral HPLC method is used to control impurities.

Batch analysis data provided for lots manufactured according to the commercial synthesis process at 2 different sites, including the commercial synthesis site, confirm satisfactory compliance and uniformity with the proposed specifications.
In addition, analytical results of all relevant batches used in non-clinical and clinical testing have been provided in order to guaranty the qualification of the impurities at the level proposed. No significant differences between lots obtained by the 2 synthesis processes have been noted, especially in terms of impurity profile.

- Stability

Stability data have been provided for 3 batches. Under accelerated conditions (40°C/75% RH - commercial packaging) and long-term conditions (25°C/60%% RH - commercial packaging), respectively 6-month and 2-year data have been provided. The photostability study performed did not show any significant change in the main stability indicating parameters.
A retest period of 2 years is supported by the presented data when palonosetron is stored in double low-density polyethylene (LDPE) stored in HPDE drums.

2.2 Medicinal product

- Pharmaceutical Development

During development studies, palonosetron showed instability in aqueous solution directly related to the product strength (see active substance), which appeared to be the most critical parameter in the product's performance. As a consequence, a low concentration of palonosetron (i.e. 0.05 mg/ml) has been selected while maintaining the required clinical dose and a low injectable volume (5ml).
Palonosetron being freely soluble in water, water for injections is used as vehicle. The pH selected for the commercial formulation ensures optimal stability of palonosetron and it is maintained over the

shelf life of the product by a citrate buffer system. Mannitol has been chosen over sodium chloride as an isotonicity agent based on superior stability of the mannitol-containing product. Palonosetron being sensitive to oxidation, a chelating agent (i.e. disodium edetate) is included in the formulation. All the excipients are of PhEur quality.
The type I glass vials and the butyl rubber stopper used as primary packaging material meet the PhEur requirements and satisfactory specifications are used to control the dimethicone used to treat the stoppers. A study has been carried out to determine the compatibility of the product with the container closure system, which is suitable for terminal sterilisation. A development study has evaluated potential extractables from the stopper.
Regarding the TSE risk, Aloxi does not contain any component of ruminant origin.

Tow different formulations were used in clinical trials. Phase I and II clinical trials used mostly a formulation based on a phosphate buffer, and containing sodium chloride as tonicity adjuster but no disodium edetate. The commercial formulation was used in all phase III studies.
Both formulations were administered as aqueous solutions by intravenous (i.v.) bolus over 30 seconds. The bioequivalence of these 2 formulations is therefore self-evident and a bioequivalence study was considered not necessary.

- Manufacture of the Product

The manufacturing process is a standard process comprising the following operations: dissolution of the active ingredient and excipients in water for injections, pH adjustment, sterile filtration, aseptic filling, stoppering and terminal sterilisation. In order to minimise any potential degradation of palonosetron by oxidation, the water for injections is continuously sparged with nitrogen during the dissolution step and a nitrogen blanket is maintained over the vessel. In-process controls and corresponding specification have been adequately defined where appropriate at each stage.
Satisfactory validation data have been provided. The conditions of the terminal sterilisation comply with the PhEur requirements and have been validated.

- Product Specification

The finished product specification include tests for appearance (PhEur), container appearance, volume in container (PhEur), identity (HPLC Rt and HPLC UV), assay (HPLC), impurities (chiral HPLC and non-chiral HPLC), pH (PhEur), sterility (PhEur), bacterial endotoxins (PhEur), container integrity and particulate matter.

Batch analysis data for 5 pilot-scale batches (3 manufactured at the commercial site and 2 manufactured at a development site), have been provided, comply with the specifications and indicate consistent and reproducible manufacture.

- Stability of the Product

Stability data have been provided for 3 primary stability batches. Under accelerated conditions (40°C/75% RH – commercial packaging – stored in upright and inverted position) and long-term conditions (25°C/60% RH - commercial packaging – stored in upright and inverted position), respectively 6-month and 30-month data have been provided. A photostability study has been performed. It showed that the product is light sensitive and that the carton used as secondary packaging provides adequate protection from light.
The results presented support the proposed shelf life and storage conditions defined in the SPC.

Discussion on chemical, pharmaceutical and biological aspects

The active substance is well characterised and documented. The pharmaceutical form selected is adequate taking into account the properties and the stability of the active substance. The excipients are commonly used for this kind of formulation and the packaging material is well documented. The manufacturing process enhances to obtain reproducible finished product batches. Stability tests under ICH conditions indicate that the product is stable for the proposed shelf life.

At the time of the CHMP opinion there was a minor unresolved quality issue which had no impact on the benefit/risk profile. The applicant committed to provide the necessary information as a follow-up measure within an agreed timeframe, and to submit variations if required following the evaluation of this additional information.

3. Non-clinical aspects

Introduction

The pivotal toxicology studies were performed in compliance with Good Laboratory Practice, which was not the case for most of the pharmacology, metabolism, dose range finding studies nor for the pilot immunohistochemical study PALO-02-06.

Non-clinical studies were conducted *in vitro*, and in rats, guinea pigs, cats, ferrets, dogs, and monkeys. Although the proposed indication for palonosetron was for short-term intravenous use only, the toxicological development has included chronic repeat dose intravenous toxicology, oral carcinogenicity testing, and reproductive toxicity studies. Juvenile toxicity studies have also been performed in rats and dogs.
No formal scientific advice was given by the CHMP on the non-clinical aspects for this application.

Pharmacology

- Primary pharmacodynamics (in vitro/in vivo)

In vitro studies

- 5-HT_3 receptor binding
Three *in vitro* studies were performed to investigate the bioreceptor binding profile of palonosetron.
The first *in vitro* study was carried out to assess the ability of palonosetron to inhibit binding of radioligands to 5-HT receptors as well as 23 additional mammalian bioreceptors. The results of this series of binding assays revealed that palonosetron possesses a strong and highly selective affinity for the 5-HT_3 receptor (pK_i = 10.4 and $pK_i \leq 5.9$, for 5-HT_3 and for other bioreceptors, respectively).
A second *in vitro* study was conducted to assess the *in vitro* saturation, competition and binding kinetic analysis of [^{3}H]-palonosetron. The affinity of [^{3}H]-palonosetron was generally 10 to 20 fold higher than [^{3}H]-granisetron.
The last *in vitro* study was carried out to analyse the distribution of palonosetron binding sites in mouse and rat brain exposed to [^{3}H]-palonosetron. The quantitative autoradiographic analysis revealed a high level of radiolabelled ligand binding in anatomical areas known to contain 5-HT_3 receptors and to be associated with antiemetic action of other 5-HT_3 antagonist.

- 5-HT_3 receptor activity
The assessment of the activity of palonosetron at 5-HT_3 receptors was tested on isolated guinea pigs ileal strips. Palonosetron acted as an insurmountable 5-HT_3 antagonist with apparent affinity (-log K_B) of 8.4 - 8.9. When tested for activity at the 5-HT_1, 5-HT_2 and 5-HT_4 receptors, palonosetron at a 1000 fold higher concentration than used in the 5-HT_3 receptor assay did not shown any effect.

- Activity of Metabolites M4 and M9 at 5-HT_3 receptor
A study has been performed to assess any possible antagonistic activity of M4 (0.3-30µM) and M9 (1-100µM), two main metabolites found in humans (see clinical pharmacokinetics), at 5-HT_3 receptors in isolated guinea pig ileum. In comparison to palonosetron (0.003-0.3µM), the two metabolites M9 and M4 demonstrated at least a 100-fold lower antagonistic activity at the 5-HT_3 receptor.

In vivo studies

- Effect on the bradycardic response to 2-methyl-5 hydroxytryptamine (2-methyl-5-HT)

A bolus intravenous injection of serotonin to anaesthetised rats induces a vagally-mediated reflex bradycardia (von Bezold-Jarisch reflex). When induced by 2-methyl-5-HT, this reflex is specifically mediated by 5-HT_3 receptors and can be blocked by 5-HT_3 antagonists. Palonosetron was evaluated for its ability to inhibit this reflex in rats and cats following intravenous, intraduodenal or dermal administration.

Palonosetron-dosed animals were administered intravenously a dose of 2-methyl-5-HT that reduced heart rate by approximately 200 beats/minute (usually 10–80 µg/kg, i.v. in rats). The cumulative dose of the 5-HT_3 antagonist causing a 50% inhibition of bradycardia was 0.02µg/kg.

The intravenous administration of single doses of palonosetron (0.01–10 µg/kg) produced a dose-related inhibition of 2-methyl-5-HT-induced bradycardia in rats. The duration of the inhibition was also dose-related. At 0.1 µg/kg palonosetron inhibited the induced bradycardia by 83% whereas administration of 1 or 10 µg/kg caused complete inhibition [16].

- Antiemetic effect

The antiemetic effect of intravenously and orally administered palonosetron was assessed in male and female dogs and in male ferrets, challenged with intravenous injections of antineoplastic agents. Antineoplastic agents employed in these experiments included cisplatin (3 mg/kg in dogs, 10mg/kg in ferrets), dacarbazine (30 mg/kg in dogs), mechlorethamine (0.4 mg/kg in dogs) and actinomycin D (0.15 mg/kg in dogs) [16]. In addition, non-chemotherapeutic emetic trials with apomorphine and copper sulphate were performed in dogs.

In dog, palonosetron (0.3-100 µg/kg, i.v. and 1-100 µg/kg , p.o), ondansetron (3-300 µg/kg, i.v. and 10-100 µg/kg, p.o) or vehicle were administered 2 hours prior to the injection of the antineoplastic agent and animals were observed for five hours. Both palonosetron and ondansetron reduced the emetic responses to all three antineoplastic agents. The antiemetic effects of both drugs were generally dose-related and when administered orally, palonosetron was about 30-fold more potent than ondansetron against each of the emtogenic agents in this animal model.

Another study was carried out in dogs who received intravenous doses of palonosetron, ondansetron or granisetron (0.1, 0.15, or 0.04 mg/kg respectively), or vehicle control 12, 10, 8, 6, 4, 2 and 1 hour prior to the intravenous injection of 3.0 mg/kg of cisplatin. Palonosetron showed some antiemetic activity when administered as long as 10 hours before the injection of cisplatin (9.83 emetic episodes *versus* 13.83 and 23 for ondansetron and granisetron respectively), but did not protect when administered 12 hours before cisplatin.

In a further study, palonosetron and ondansetron were assessed for their relative abilities to reverse cisplatin-induced emesis. In these experiments dogs received intravenous injections of palonosetron, ondansetron or vehicle one hour after the intravenous administration of 3.0 mg/kg cisplatin. Each animal was observed continuously for five hours following cisplatin administration to evaluate the number of emetic episodes. Palonosetron was more potent than ondansetron.

An experiment was conducted to study the relationship between plasma concentration of palonosetron and protection of dogs against cisplatin-induced emesis. Groups of dogs received oral doses of palonosetron (0, 100, 316, or 1000 µg/kg) or vehicle control 30 minutes prior to the injection of cisplatin. Plasma concentrations of palonosetron were determined by an HPLC-radioimmunoassay method at 0, 0.25, 0.5, 1, 2, 4, 8, 24, and 48 hours after the administration of palonosetron and systemic exposure was expressed as $AUC_{(0\text{-}4\ hr)}$. Systemic exposure to palonosetron, as estimated by computed $AUC_{(0\text{-}4hr)}$ values, were approximately dose-proportional over the range studied. However, a relationship between systemic exposure and magnitude of antiemetic effect could not be demonstrated. Dogs dosed with palonosetron had significantly fewer emetic episodes than vehicle control animals but there was no evidence of a significant dose-response relationship.

In male ferrets, the use of palonosetron to antagonise the emetic effect of 10.0 mg/kg cisplatin i.v. showed that palonosetron administered intravenously resulted in a significant reduction in emetic episodes at all of the doses tested (0.001 to 0.1 mg/kg). Doses of palonosetron of 0.003 mg/kg or higher were significantly effective in ferrets [16].

- Secondary pharmacodynamics

Secondary pharmacodynamic interaction studies were conducted for 5-HT_1, 5-HT_2 and for NK-1 receptors.

In one study (AT 6446) substance P-induced contractions of guinea pig ileum was slightly reduced by palonosetron (1 μM), albeit at concentrations 1000 times those used to elicit 5-HT_3 antagonistic responses.

Activity at 5-HT_4 receptors was investigated in a number of studies. AT5964 demonstrated that palonosetron was devoid of agonist or antagonist activity in a guinea pig hippocampal membrane assay at concentrations of 100 μM. PALO-01-30 demonstrated that contractions could not be induced by palonosetron in guinea pig proximal colon up to 100 μM, but that palonosetron was able to block the 5-methoxytryptamine (5-MeOT) induced contractions at starting concentrations of 3 μM. IC_{50} was 12 μM, whereas 5-HT_3 receptor in guinea pig ileum had an IC_{50} of 0.028 μM (400 times greater potency). The method-control antagonist substance was SDZ-205,557.

A radioligand binding assays were performed in human recombinant CHO-K-1 cells expressing 5-HT_{5A} and cells expressing NK-1 receptors and in human recombinant HeLa cells expressing 5-HT_6 receptors. Specific and non-specific ligands at 5HT receptors were LSD and serotonin respectively. Reference compounds for 5HT receptors were methiothepin. At NK-1 receptors, agonist was substance P and antagonist was L-703,606.

IC_{50} values for methiothepin inhibition of 5-HT_{5A} and 5-HT_6 were 2.9 nM and 2.8 nM. Only 10 μM palonosetron was able to inhibit the 5-HT_{5A} receptor by 23%, whereas there was a slight stimulation (4%) of palonosetron at 5-HT_6 receptors at 10 μM.
IC_{50} of L-703,606 was 0.025μM on NK-1 receptors. Palonosetron at 10μM was only able to inhibit the NK-1 receptor by 3%.
On guinea pig ileum, the reference agonist at NK-1 receptors was substance P that resulted in a 100% response at 3 nM, whereas palonosetron only resulted in 44% agonist activity at 30 μM relative to substance P response. Likewise, L-703,606 (1 μM) was used as the control antagonist which demonstrated a 66% reduction in agonist response compared to palonosetron which showed a 19% reduction in activity at 30 μM.

Discussion on secondary pharmacodynamic studies
Based on the pharmacodynamic data submitted, palonosetron was recognized to be highly selective for 5-HT_3 receptors, in line with the results of other published radioligand binding experiments [17] [18]. However, the applicant did not directly investigate secondary pharmacodynamic responses, because of the specificity of palonosetron binding to 5-HT_3 receptors, the absence of effects at sublethal doses in toxicity investigations and the absence of changes observed in safety pharmacology investigations.

- Safety pharmacology

Central nervous system
Palonosetron given orally to mice at 10 to 60 mg/kg had no significant effect on responsiveness to sub-convulsive electroshock or metrazol treatment. Intraperitoneal palonosetron in mice at up to 1 mg/kg did not influence behaviour in a two-compartment exploratory black/white box model of anxiety. In an independent experiment at a different laboratory oral treatment at up to 3 mg/kg significantly reduced the amount of time in the dark indicating a weak anxiolytic effect. In another study intraperitoneal administration to mice at up to 10 mg/kg had no meaningful effect on the central or autonomic nervous systems or on responsiveness to external stimuli. In all cases clear effects were seen with positive control substances.

Cardiovascular system
Effects on ionic currents

The effects of palonosetron on the I_{Kr} and I_{Na} currents were investigated in a series of *in vitro* studies using stably transfected HEK293 cells to examine hERG and hHNa tail currents and using isolated "M" mid-myocardial ventricular cells from dogs.
The IC_{50} result for the I_{Kr} channel was similar in three different experiments. A similar I_{Kr} IC_{50} was obtained for ondansetron (study PALO-01-01) and palonosetron inhibited the sodium current (study PALO-01-02) to a greater extent (6.5 μM) when compared to ondansetron (48.6 μM). These experiments were carried out at 3 Hz, which are likely to overestimate the block observed in the sodium current, and therefore, the IC_{50} obtained.

Effects on action potentials

Effects on action potentials were investigated in guinea pig papillary muscles, rabbit Purkinje fibres, and dog Purkinje fibres and changes in action potential duration consistent with the effects seen on I_{Kr} channels at the cellular level were noted in all three systems (studies PALO-01-32, PALO-01-33 and PALO-99-48).
Dose-related effects were statistically significant at 3 μM in all models and at 0.3μM in the most sensitive model, rabbit Purkinje fibres, under the normal stimulation rate (60 pulse/min) only. A reverse use-dependency was noted in both Purkinje fibre models. Decreases in the amplitude of action potential and in depolarisation rate were also noted at the highest concentration (30 μM), consistent with I_{Na} blockade.
The human metabolite M9, had no effect at the highest concentration tested and the intermediate 07-PALO, which is present in the finished product at not more than 1%, induced similar changes to those caused by palonosetron. In dog Purkinje fibres, ondansetron also induced similar changes at 1 μM or more (PALO-01-03).
The lowest concentration tested, 0.03μM, did not cause any changes in any model and is approximately three fold higher than the plasma concentrations seen in patients treated at the proposed clinical dose.

Effects in vivo

In an anaesthetised dog model (study AT 6242), transient minor reductions in blood pressure were observed immediately after intravenous injections of palonosetron at 0.3 or 1.0 mg/kg. There were no changes in the QT interval or in any other electrocardiographic parameter.
In a series of telemetry studies conscious dogs were given bolus injections of palonosetron (PALO-99-49), M9 or 07-PALO (PALO-01-05) or a 15-minute infusion of ondansetron (PALO-01-04). There were no meaningful changes in blood pressure or in the electrocardiograms with palonosetron at up to 1 mg/kg, the highest dose studied. There were also no changes with M9 at up to 0.1 mg/kg or with 07-PALO at up to 0.01 mg/kg. These dosages were selected to mimic up to 100-fold higher than the maximum possible human exposures. Ondansetron at 7.5 mg/kg, 15-fold greater than the maximum human dose, was associated with prolonged QT intervals, increased heart rates and changes in T-wave morphology indicating effects on ventricular repolarisation.
In anaesthetised vagotomised β-adrenoreceptor blocked guinea pigs (PALO-01-34) there were no effects at intravenous dosages of up to 1 mg/kg; at 3 mg/kg prolonged monophasic action potential was attributed to bradycardia; 10 mg/kg was fatal.
In anaesthetised α_1-adrenoreceptor activated rabbits (PALO-01-35), a pro-arrhythmic model designed for detection of Torsades de Pointes, various effects on cardiac conduction and arrhythmic episodes were seen after a 10-minute intravenous infusion of 10 mg/kg but there were no Torsades de Pointes.
In a hemodynamic and respiratory investigation in mongrel dogs (AT 5493) palonosetron caused no effects at intravenous dosages of up to 1mg/kg.

Respiratory system

The absence of hemodynamic effects in mongrel dogs (AT 5493) was confirmed in a follow-up study (AT 6161) that included extensive respiratory measures. Some marginal changes at 1 mg/kg were attributed to individual variation.

Autonomic nervous system

Intravenous palonosetron at up to 1mg/kg in dogs caused a mild to moderate dose-dependent attenuation of the blood pressure response to sympathetic ganglionic stimulation with McNeil-A-343

(AT 6168). This suggests that palonosetron had modest inhibitory activity at muscarinic receptor sites on sympathetic ganglia. Challenge with norepinephrine, isoproterenol, dimethyl-4-phenylpiperazinium and acetylcholine had no effect.

Gastrointestinal system
Palonosetron given orally to rats at up to 0.3 mg/kg had no effect on appetite over the following four hours (AT 5452). Intraperitoneal administration to rats of doses of up to 1 mg/kg had no effect on gastric emptying (AT 5244).

Renal/urinary system
In a renal function study using normotensive male rats, oral palonosetron was associated with a decrease in chloride and sodium excretion within one hour of treatment at up to 0.1 mg/kg (AT 6005). There was no effect at 1 mg/kg and these changes were not considered physiologically meaningful.

Discussion on Safety pharmacology programme
The safety pharmacology data obtained from the studies to assess the effect of palonosetron on the central nervous system, were consistent with the low affinity of palonosetron for bioreceptors other than 5-HT_3 and suggested little likelihood of centrally mediated effects.
Although convulsions, ataxia, subdued behaviour and occasional changes in gait were reported in many of the toxicity studies, in general these occurred only at fatal or near fatal dosages and probably reflect extreme physiological conditions that have no relevance to potential clinical events. Since these changes usually occurred in the period immediately after dosing, it is likely that they were associated with high plasma concentrations.
The changes observed in the *in vitro* studies to assess the cardiovascular safety of palonosetron were consistent with the known effects of 5-HT_3 antagonists, all of which inhibit both I_{Kr} and I_{Na} currents [19]. The changes occurred at concentrations that are greater than those anticipated in clinical practice and it was considered unlikely that palonosetron would have any effect on these channels in human.
Moreover, palonosetron is 60 % protein bound, and therefore, the free drug concentrations in human patients are lower than those associated with any change observed *in vitro* on the action potentials, even in the most sensitive model tested. It was considered unlikely that the observed changes in these models would have any significant safety implications for patients.
Results form safety pharmacology studies suggested little concern for effects on respiratory, gastro-intestinal, renal, central and autonomic system functions at therapeutic doses.

- Pharmacodynamic drug interactions

Since palonosetron will be co-administered with anticancer chemotherapeutic agents, a number of interaction studies using intraperitoneal injections of palonosetron have been carried out in mice. These include interactions with cisplatin (PALO-99-68, AT 6777), cytarabine (PALO-99-36), cyclophosphamide (PALO-99-37), mitomycin C (PALO-99-66) and doxorubicin (PALO-99-67), using mouse cancer models according to USA National Institute of Health protocols. Dosages of 1, 10 and 30 mg/kg of cisplatin were used on study AT 6777 and of 10 mg/kg for all other studies, repeated up to ten times. No significant interactions were observed in any of these studies.

- Summary of salient pharmacodynamic findings

Palonosetron hydrochloride is a serotonin (5- hydroxytryptamine or 5-HT) receptor antagonist which exerts its effect by interacting with the 5-HT_3 receptors as an antagonist. Palonosetron is structurally unrelated to currently available 5-HT_3 receptor antagonist. Palonosetron has shown little or no affinity for other bioreceptors, including other serotonergic receptors (5-HT_1, 5-HT_2 and 5-HT_4). The antagonist activity of palonosetron main metabolites, M4 (hydroxy derivate) and M9 (N-oxide derivate), was considered clinically non-relevant.

In vivo studies to assess the antiemetic effect of palonosetron were carried out in rat, dog and ferret including comparison with the antiemetic drugs granisetron and ondansetron. Outcomes revealed that palonosetron was able to reverse the emetic effects of cisplatin, dacarbazine, actinomycin-D and

mechlorethamine. The antiemetic effect of palonosetron was shown to be more potent and more prolonged in time than that of granisetron and ondansetron.

The safety pharmacology of palonosetron including central nervous system, respiratory system, autonomic nervous system, gastrointestinal system, renal/urinary system, blood compatibility and hemodynamic and respiratory effects have been studied. Moreover, extensive investigation of cardiovascular safety was performed. In vitro studies confirmed the expected effects of palonosetron, which are those of the other 5-HT_3 antagonists, on I_{Kr} and I_{Na} currents and action potentials, but at very high concentrations. *In vivo* studies using several species showed effects on cardiac conduction, but no Torsades de Pointes were observed, despite the use of doses up to 1 mg/kg (which is 300-fold higher than the proposed human dose).
The applicant provided adequate arguments to justify why oral studies sufficiently describe the effects of palonosetron on gastro-intestinal, renal and CNS functions regardless of route of administration.

The CHMP considered the non-clinical pharmacodynamic drug interaction studies performed to investigate possible effects on tumour pathology not sufficient, as they did not address possible adverse interactions.

Pharmacokinetics
The pharmacokinetics at low dosages have been studied in rats (albino and pigmented strains), dogs, monkeys and human using labelled [^{14}C]-palonosetron. The animal studies included both intravenous and oral routes of administration. Only the intravenous route has been investigated in humans since this is the proposed route of clinical administration. Some further studies, using higher oral dosages of cold material and validated analytical methods, have been carried out in rats and dogs.

- Method of analysis

A number of validated methods were used. An HPLC method with radioimmunoassay quantification was developed for palonosetron and M9 (IAR-B-1009), and was then supplemented with a UV absorbance detection method for higher concentrations (IAR-B-1013). Subsequently three LC/MS/MS methods were developed and validated for quantitation of palonosetron and M9 in mouse, rat and dog plasma and were used in conjunction with toxicology and carcinogenicity studies (those starting with the code PALO-).

- Absorption-Bioavailability

Absorption was high in rats, dogs and monkeys, as deduced from the predominant urinary excretion of radioactivity, which showed little difference between oral and intravenous administration. Absorption was rapid in rats and dogs following oral administration. T_{max} in rats was at 0.25 hours, the first sample collected, regardless of dose and at 0.375 hours in dogs treated at 20 mg/kg but around 1 hour following treatment at 0.5 mg/kg. In monkeys treated at 0.5 mg/kg, T_{max} was at 8 hours.

Despite high and rapid absorption, the oral bioavailability of palonosetron was low in all three animal species examined (6.4, 12.5 and 9.0% in rats, dogs and monkeys, respectively). The data suggested that absorption of radioactivity in these studies was around 100% and the low bioavailability was attributed to extensive pre-systemic metabolism.

- Distribution

The single dose plasma kinetics of palonosetron derived from various studies performed in albino and pigmented rats, dog and monkey (excluding toxicokinetic data) were compared (see table 1):

Table 1: Plasma kinetic parameters of palonosetron following single administration of [^{14}C]-palonosetron or palonosetron:

Species	Dose [a] (mg/kg)	C_{max} (ng-Eq/mL or ng/mL)	$AUC_{(0\text{-}tlast)}$ [d] (ng-Eq·h/mL or ng·h/mL)	$T_{1/2}$ (h)	T_{max} (h)	CL ($L.hr^{-1}.kg^{-1}$)	Vdβ (L/kg)	Report
Intravenous administration								
Rat (albino)	0.5	58.3	58.9 ^{24h}	1.49	0.083	7.96	17.1	AT 6264
Rat (pigmented)	0.5	52.9	51.9 ^{24h}	0.877	0.083	8.67	11.0	AT 6285
Dog	0.5	122	194 ^{24h}	1.87	0.083	2.58	6.95	AT 5976
Monkey	0.5	112	495 ^{48h}	4.44	0.083	1.03	6.60	DM 1078
Man	0.010	3.13	65 $^{\infty}$	37.4	0.083	0.16	8.34	2216
Man	0.003 [c]	5.63	8.57 ^{24h} / 35.8 $^{\infty}$	56.4	0.144	ND	ND	2330
Oral administration								
Rat (albino)	0.5	0.964	3.94 ^{24h}	ND	0.25	ND	ND	AT 6264
	0.5 [b]	0.346	0.454 ^{4h}	ND	0.25	ND	ND	AT 6303
	60 [b]	2170	2520 ^{8h}	ND	0.25	ND	ND	AT 6303
	180 [b]	5600	11300 ^{24h}	ND	0.25	ND	ND	AT 6303
Dog	0.5	16.4	24.2 ^{48h}	2.17	1	ND	ND	AT 5976
	0.5 [b]	4.98	29.9 ^{48h}	2.12	1.38	ND	ND	AT 6304
	6 [b]	100	295 ^{48h}	4.54	0.500	ND	ND	AT 6304
	20 [b]	1400	2890 ^{96h}	9.97	0.375	ND	ND	AT 6304
Monkey	0.5	4.65	44.4 ^{48h}	ND	8	ND	ND	DM 1078

a Based on [^{14}C]-palonosetron except:
b palonosetron; samples analysed using validated methods IAR-B-1009 or IAR-B-1013;
c palonosetron, at the proposed clinical dose, samples assayed using validated method IAR-B-1058.
d except man, presented as (0-24) or (0-∞). Time of last data indicated by superscript.
Abbreviations: AUC = area under the concentration versus time curve, C_{max} = maximal observed plasma concentration, CL = clearance, ND = not determined, $T_{1/2}$ = half-life, , Vdβ = volume of distribution where β is the terminal elimination rate constant.

No differences were noted between albino and pigmented rats. In all animal species, the C_{max} and AUC values following oral dosing were considerably lower than after intravenous administration at the same dosages, consistent with extensive first-pass metabolism.
The C_{max} and AUC at high oral dosages in rats were considerably greater than predicted by the data at 0.5 mg/kg, indicating that elimination mechanisms may be saturated although palonosetron was still rapidly cleared from the plasma. The plasma kinetics in dogs treated orally at 20 mg/kg, C_{max} and to a lesser extent AUC were greater than predicted by the 0.5 mg/kg data, suggesting a similar saturation of elimination mechanisms.
Both the clearance and volume of distribution following intravenous treatment in rats were higher than in dogs.
The plasma kinetics in monkeys were considerably closer to those of dogs than to humans. Compared with dogs, monkeys have a slightly longer half-life and slower clearance following intravenous treatment and consequently higher AUC, together with slow absorption following oral administration (T_{max} at eight hours), and a lower C_{max} (see clinical pharmacokinetics).

Following repeated oral administration for five days at 60 mg/kg in rats (AT 6302) or at 6 mg/kg in dogs (AT 6301), there was very little difference in the plasma kinetics of palonosetron compared with Day 1 (see table 2).

Table 2: The plasma kinetic parameters of palonosetron following multiple oral administration

Species	Dose level (mg/kg/day)	C_{max} (ng/ml)	$AUC_{(0-24hr)}$ (ng·hr/ml)	$T_{1/2}$ (hr)	T_{max} (hr)	Reports
DAY 1						
Rat	60	1970	3340	ND	0.25	AT 6302
Dog	60	126	519	1.74	1.56	AT 6301
DAY 5						
Rat	60	2030	2490	ND	0.5	AT 6302
Dog	60	175	659	2.06	0.813	AT 6301

Abbreviations: AUC = area under the concentration versus time curve, Cmax = maximal observed plasma concentration, CL = clearance, ND = not determined, T1/2 = half-life, Tmax= time at which maximal concentration achieved, Vdβ = volume of distribution where β is the terminal elimination rate constant.

Tissue distribution studies in albino rats following intravenous administration of single doses of [^{14}C]-palonosetron at 0.5 mg/kg (AT 6264) indicate tissue to plasma ratios that were generally greater than one. Highest ratios were in the intestinal tract, consistent with biliary excretion. High ratios were also apparent in the kidney and bladder, consistent with urinary excretion. Apart from these tissues, highest concentrations were apparent in lungs, liver, adrenals and testes and lowest in fat, bone (without marrow) and the medulla (brain). Concentrations generally peaked within two hours, later peaks were observed in skin, testes, liver, stomach, bone and fat. Concentration ratios in the brain peaked at 30 min; chromatographic analysis indicated only palonosetron, no metabolites. The kinetic pattern in the brain mirrored that in plasma, indicating rapid elimination. By 96 hours, concentrations in all tissues, except the eye, were below the limit of quantitation.

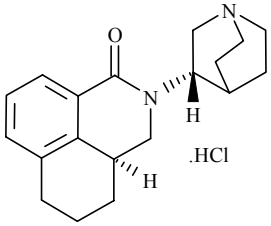
In a pigmented rat study (AT 6285) similar results were obtained except that concentrations of radioactivity were highest in the eye and high concentrations persisted at 96 hours. The half-life of elimination from pigmented eyes was calculated to be 4.71 days indicating that palonosetron or one of its rat metabolites has potential for reversible melanin binding.

An *in vitro* study demonstrated non specific and non saturable binding of palonosetron to plasma proteins (CL 6204). Binding was approximately 48, 66 and 62% in rat, dog and human plasma.

- Metabolism (in vivo/in vitro)

Fourteen different metabolites have been detected in rats, dogs and monkeys, of which eight have been structurally identified. The general metabolic reactions are oxidation at the nitrogen in the hetero-bicyclic ring to produce an N-oxide and the addition of hydroxy groups to the molecule. In some cases the hydroxy groups are further oxidised to the corresponding ketone (see figure 1).

Figure 1: Structural formula of palonosetron



Molecular Formula: $C_{19}H_{24}N_2O$ HCl
Mr: 332.87.

The main human metabolites, M4 and M9 are also produced by rats, dogs and monkeys. Two other metabolites, M5 and M6, have been identified at trace levels in human plasma; both are produced in dogs but only M6 has been found in rats.

The major metabolites were M1 and M12 in rat plasma, M1, M2 and M3 in dog plasma. None of these have been identified in human. In monkey M8 was the major metabolite and M4 and M9, each accounted for more than 10% of radioactivity in the urine. In each species, palonosetron was a significant component of activity in plasma but accounted for less than 6% of activity in urine.
Following oral treatment the metabolite patterns in rats and dogs were similar to those seen after intravenous dosing.
Small but measurable amounts of M4 were detected in rat plasma following both oral and intravenous treatment and there was no difference between pigmented and albino animals. Larger amounts were found in both dogs and monkeys together with significant amounts of M9, regardless of the route of administration. M9 was not detected in rats following treatment with [^{14}C]-palonosetron at 0.5 mg/kg, but was apparent in significant amounts following oral treatment at higher dosages (AT 6303). In monkeys, the oral T_{max} for both metabolites was eight hours, as for palonosetron, and four hours for M4 following intravenous dosing. In all other cases T_{max} was at two hours or less. The longest half-life, of those determined, was for M4 in dogs at 4.26 hours.
Following repeated administration to rats and dogs, the exposure to M9 was higher than following single doses (AT 6301 and AT 6302).

There have been no *in vitro* or *in vivo* animal studies of enzyme induction. However, studies have been carried out using human biomaterials and are discussed in the clinical section.

- Excretion

The major route of excretion in rat, dogs and monkeys was urinary (further to oral and i.v. administration). Biliary excretion was significant in rats and to a lesser extent in dogs. In rats most of the dose was recovered within the first 24 hours. In dogs most of the dose had been recovered within 48 hours. Excretion in monkeys was essentially complete by 72 hours.

- Pharmacokinetic drug interaction

Potential pharmacokinetic interactions have not been investigated as part of the nonclinical development.

Toxicology

The hydrochloride (HCl) salt and free base forms have been administered in non-clinical studies. Most studies used the hydrochloride salt. The intended clinical i.v. dose of palonosetron (0.25mg) corresponds to approximately 0.003 mg/kg.

- Single dose toxicity

Studies were carried out in mouse, rat and dog using intravenous and oral route of administration. Death in all species was associated with convulsions and collapse. According to the results of the experiments there were no effects on body weight or food intake in any study. There was no indication of any influence of sex on the outcomes.
Single dose toxicity studies established a maximum non-lethal intravenous dosage of 10 mg/kg in rats and mice and 20 mg/kg in dogs. A maximum non-lethal oral dosage of 250 mg/kg in rats, 100 mg/kg in mice and 50 mg/kg in dogs were established. Signs seen at non-lethal dosages included inactivity, tremors, ataxia and laboured respiration.

- Repeat dose toxicity (with toxicokinetics)

A summary of the repeat dose toxicity studies carried out in mouse, rat and dog is provided in table 3.

Table 3: Summary of the repeat dose toxicity studies

Species	Route	Duration	Group size m + f	Dosages (mg/kg/day)		Report
				Tested	NOAEL	
Mouse	Oral gavage	3 months	10 + 10	0, 30, 60, 90, 120	60	AT 6751
	Oral gavage	10 months	75 + 75	0, 0, 10, 30, 60	NR	AT 7464
Rat	Intravenous	1 month	10 + 10	0, 1, 3, 10	10	AT 5962
		26 weeks	20 + 20 [a]	0, 2, 7, 10	7	PALO-99-08
	Oral gavage	1 month	10 + 10	0, 6, 18, 60, 180	18	AT 6329
		3 months	15 + 15	0, 18, 60, 120, 180	18	AT 6665
		15 months	75 + 75	0, 0, 15, 30/45, 60/90	NR	AT 7455
Dog	Intravenous	up to 10 days	2 + 2	5, 10, 15	5	PALO-99-19
		1 month	3 + 3	0, 1, 3, 10	3	AT 5963
		9 months	4 + 4 [b]	0, 1, 3, 6	6	PALO-99-10
	Oral gavage	2 weeks	1 + 1	0, 10, 20, 40	10	708-D-92
		1 month	3 + 3	0, 2, 6, 20	6	AT 6328
	Oral capsule	3 months	5 + 5	0, 2, 10, 40	10	AT 6787

Abbreviations: [a]: an additional 10 +10 were allocated to the control and high dose group for recovery studies; [b]: an additional 2 + 2 were allocated to the control and high dose group for recovery studies; f = female; m: male; NR: not relevant; NOAEL: no observed adverse effect level.

Intravenous studies

In a 26-week rat study, the highest dosage was originally scheduled to be 14 mg/kg/day but this was reduced after a single dose because of the severity of signs observed. Increased mortality was apparent at 10 mg/kg/day on this study (six rats, including three toxicokinetic satellites). A no observed adverse effect level (NOAEL) of 7 mg/kg/day was established.

A high dose regimen was planned on a nine-month study in dogs but severe signs (convulsions, ataxia, subdued behaviour and emesis) and one death forced a dosage reduction after the second day at 10 mg/kg/day. There was no toxicity observed at 6 mg/kg/day.

The treatment-related clinical signs observed at the highest dosages included convulsions, ataxia, exaggerated startle response, tremors, inactivity, transient vocalisation and various changes in gait or stance. There were no effects on body weight, food intake, ophthalmoscopy, electrocardiography, clinical pathology, macroscopic pathology, organ weights or histopathology. Exposures, in terms of AUC, at the no observed adverse effect levels in the chronic studies was at least 170-fold higher than observed in human patients.

Oral studies

In a 3-month mouse study, mortality was increased in males at 90 mg/kg/day and in both sexes at 120 mg/kg/day, with associated clinical signs. Urinary protein output was increased in females at 90 mg/kg/day and 120 mg/kg/day and accessory sex organ weights were reduced in males at 120 mg/kg/day.

In a one-month rat study changes at 180 mg/kg/day included reduced body weight gain, occasional signs and numerous clinical pathology variations, most of which were within the normal range. Low testis and secondary sex organ weights were associated with degeneration and necrosis of the seminiferous epithelium. Low thymus weights in females associated with lymphoid depletion and high liver weights in both sexes with foamy cytoplasmic swelling in centrilobular hepatocytes. The

only potentially significant change that extended to lower dosages was high adrenal weights, seen in males treated at 18 mg/kg/day or more. This was not associated with any histopathological change.
Mortality was clearly increased at 180 mg/kg/day in the three-month study; most males died. Histopathology changes included reduced trabecular bone, testicular atrophy, lymphoid atrophy primarily in the spleen, progressive nephropathy, decreased bone marrow cellularity, increased thymic atrophy, hypertrophy of the adrenal zona glomerulosa and increased height of the thyroid follicular epithelium. There were no treatment-related deaths at 120 mg/kg/day but similar histopathology changes were seen in the bone, testis, spleen and kidney. Treatment related changes at 60 mg/kg/day comprised low body weights in males, occasional signs, various minor clinical pathology changes, increased liver and spleen weights and, in a few males, histopathological changes in bone and testis. Minor clinical pathology changes at 18 mg/kg/day were not considered significant.
Exposure to palonosetron at 60 mg/kg/day was more than 170-fold higher than in patients. Data from the study PALO-98-03 suggest that the repeat dose oral exposure at 15 mg/kg/day was 30 to 50-fold higher than in patients.

In a 3-month dog study, once daily treatment at 40 mg/kg was associated with convulsions, weight loss, and thymic atrophy. Treatment at 20 mg/kg once daily or 10 mg/kg twice daily was associated with low alkaline phosphatase and cholesterol levels. Treatment at 20 mg/kg once or twice daily was associated with low testis weights.

- Genotoxicity in vitro and in vivo (with toxicokinetics)

- Three *in vitro* studies were conducted.
A bacterial reverse mutation test (Ames test) was conducted. Doses tested were from 33 to 1000 and 5000 µg/plate, with or without metabolic activation (S9 mix). There was no evidence for mutagenic activity.
A mammalian cell mutation (CHO/HGPRT assay: Chinese hamster ovary/hypoxanthine-guanine phosphoribosyl transferase) was conducted. Doses tested ranged from 300 to 800 µg/ml without metabolic activation (S9 mix) and from 200 to 1000 µg/ml with activation. There was no evidence for mutagenic activity of palonosetron.
A chromosome aberration assay was conducted in CHO cells. Doses tested ranged from 151 to 355 µg/ml without S9 mix and from 277 to 650 µg/ml with S9 mix. A clastogenic effect was observed in the absence of metabolic activation and an equivocal response with metabolic activation.
An additional *in vitro* photo-chromosome aberration assay performed in V79 cells, was negative.
- Two *in vivo* studies were conducted.
A mouse micronucleus assay was conducted. Mice were treated intravenously at up to 10 mg/kg, there was no evidence for mutagenic or clastogenic effects.
Palonosetron was also tested in the *in vivo* Unscheduled DNA Synthesis test in rat hepatocytes. Rats were treated intravenously at doses of up to 30 mg/kg. There was no evidence for DNA damage.

- Carcinogenicity (with toxicokinetics)

Two long-term carcinogenicity studies were performed in rat and mice. In rats, a 104 weeks oral gavage study was conducted at daily doses from 0 to 60 mg/kg/day in male rats and from 0 to 90 mg/kg/day in female rats. In mice, a 104 weeks study was also conducted in which animals were exposed to palonosetron by oral gavage in concentrations of 0 to 60 mg/kg/day.

Oral dosages used were multiples of the proposed human dosage (based on $AUC_{0\text{-}24h}$) ranging from 136 to 1220-fold in males and from 61 to 706-fold in females.

In mouse, macroscopic examination indicated an increased incidence of swollen spleen in males treated at 15 mg/kg/day and in females treated at 60 mg/kg/day. These observations were not associated with any significant histopathological change. There was no increase in the incidences of any tumour in any group of female mice. The only significant increase in tumours among male mice was for the combined incidence of malignant lymphoma and malignant pleomorphic lymphoma at the lowest dosage of 15 mg/kg/day. There was no increase in this tumour type among animals killed at the terminal sacrifice.

In rat there were increased incidences of proliferative lesions at all dosages in the pancreas and pituitary of males and, at the high dosage only, in the adrenals, liver, thyroids, mammary gland, skin and tail. At examination after 104 weeks, the erythrocyte count was reduced in rats treated at 45 mg/kg/day or more. Neutrophil counts were slightly increased in females treated at 45 or 90 mg/kg/day. Changes in pituitary, adrenal, liver and spleen weights were also seen at the intermediate dosages and increased liver weight was considered to extend to rats treated at 15 mg/kg/day. The incidence of clear cell foci in the liver was also seen in males treated at 15 mg/kg/day. Degeneration of the tubular germinal epithelium of the testis was seen in almost all males treated at 60 mg/kg/day, the change was severe in most cases and accompanied by hypospermia in the epididymides; the incidence and severity of these changes was unaffected at lower dosages.
Males at all dosages had a higher incidence of keratoacanthoma than in control males. In addition males treated at the high dosage had a high incidence of squamous cell papillomas on the tail. These tissues were only examined if macroscopically abnormal. Females were unaffected.

Discussion on carcinogenicity
With the exception of a small increase in hepatocellular adenomas in female rats, the increase in the incidences of tumors were predominantly those of the endocrine system for which the rat is known to be particularly susceptible.
There is interaction of 5-HT_3 antagonists with inhibition of dopamine activity. Activation of postsynaptic 5-HT_3 receptors located on dopaminergic terminals may lead to changes in dopamine transmission in *in vivo* experiments performed in rats [20]. 5-HT_3 receptor antagonists partially inhibit, with low potency, the stimulation of dopamine release[21]. Ondansetron is able to attenuate increases in dopamine activity [22]. In conclusion, there are links between the serotonin and dopamine systems, especially noted in high doses where selectivity of receptors would be less likely, which explain endocrine tumour development.
Increases in liver weights were only noted in high dose animals (above 60 mg/kg/day) and were not associated with any significant pathology changes. The increased liver weights may have been caused by hypertrophy owing to the extensive drug metabolism. Most of the increased liver weights were associated with increases in clear cell foci, which refer to glycogen and possibility of altered metabolism. The studies in which these changes were noted were carcinogenicity studies, and liver weight increases were only apparent after a life-time of oral administration to rats. These effects were unlikely to be of relevance to human, clinical human exposure being a single intravenous administration.
Based on historical histopathology data on selected tumours in rats, the apparent increase of keratoacanthomas in male rats treated with palonosetron was not ascribed to treatment with palonosetron.
The AUC achieved in these studies were much higher than the exposure achievable in man in single dose administration, and therefore the potential risk to humans was not considered relevant.
Also, controls had unusually low incidences of the tumours, this may have been the reason for statistically significant results in these studies. As exposure was so much higher and treatment was for so long a period compared to the intended human administration dose and regimen, it was concluded that the risk these tumours pose for humans is not relevant.

- Reproductive and developmental studies

Reproduction studies were summarised table 4.

Table 4: Summary of Reproduction Toxicity Studies

Species	Route	Treatment period	Group size m + f	Dosages, mg/kg/day		Study names
				Tested	NOAEL [a]	
Rat	i.v.	2w pre-mating + 4w	10 + 0	0, 1, 3, 10	10	AT 6267
	Oral	61-63d pre-mating	15 + 0	0, 18, 60, 120	60	AT 6700
		2w pre-mating to Day 9 gestation	0 + 30	0, 9, 30, 60	30	AT 6750
Rat	Oral	Days 7-16 gestation	0 + 9	0, 30, 90, 180	90	703-R-94
		Days 7-16 gestation	0 + 20-24	0, 18, 60, 120	18	AT 6756
Rabbit	Oral	Days 7-19 gestation	0 + 6	0, 10, 30, 100	30	704-B-94
		Days 7-19 gestation	0 + 8-17	0, 18, 60, 120-90 [b]	120-90	AT 6755
Rat	Oral	Day 6 gestation to Day 21 *post partum*	0 + 24	0, 9, 30, 60	60	PALO-99-13

Abbreviations: [a] For reproduction effects; [b] Dosage reduced because of maternal deaths; w: weeks.

Fertility and embryonic development

Based on the results of the male rat fertility studies, there were no changes at 60 mg/kg/day associated with treatment. In the female rat oral study there was a small but statistically significant reduction in the number of females treated at 60 mg/kg/day that mated, with a consequent reduction in the number of pregnancies.

Embryo-foetal development

In rats, no significant malformations of foetus were identify and there was no change in the incidences of visceral anomalies. Skeletal examination indicated reduced ossification, consistent with the reduction in foetal body weights, particularly at the highest dosage. There was no indication of any other morphological change. In rabbits, there were no treatment-related changes in clinical signs, body weight gain or necropsy observations. There was no effect on the numbers of corpora lutea, implantations, resorptions or live litter size. There was no significant effect on foetal body weight, sex ratio or on the incidences of external, visceral or skeletal malformation or variations.

Prenatal and postnatal development, including maternal function

Pregnant rats were orally treated (at doses from 0 to 60 mg/kg/day) from day 6 of gestation to weaning of their offspring on day 21 *post partum*. There was evidence of maternal toxicity at 60 mg/kg/day during the early lactation phase and two dams were sacrificed following the death of their litters. There was no effect on reproduction in the F1 generation.

Studies in the offspring

Neonatal rats were treated at 0 to 50 mg/kg/day subcutaneously from day 4 post partum for 8 to 31 days, and dogs were treated at 0 to 6 mg/kg/day intravenously from approximately two weeks of age for 14 to 28 days. These studies did not show any toxicity that was not apparent in adult animals.

Discussion on reproductive and developmental studies

Although oral treatment of rats (one-month repeat-dose toxicity study in rat) was associated with degeneration of the seminiferous epithelium, this was not observed in i.v. fertility studies, leading to the conclusion that this toxic effect might be due to a metabolite. No treatment-related teratogenic effects were seen. Maternal toxicity was the limiting factor in the embryo-foetal studies. Palenosetron did not affect pre- or postnatal development, except at high oral doses, unrepresentative of the clinical situation.

The selection of the oral gavage and subcutaneous routes used in the majority of the reproduction toxicity studies has been adequately justified by the applicant.

The CHMP considered the studies conducted acceptable and the NOAELs were high enough to allow a reasonable assumption of safety in human.

- Local tolerance

Intravenous irritation studies performed in rabbits using a high concentration (10 mg/ml) of the clinical formulation of palonosetron were negative. There was no indication of increased irritation at the injection sites in either of the chronic rat and dog studies, which involved repeated daily intravenous injections for six or nine months.

Discussion on local tolerance
Considering that the chronic toxicity studies were performed with a different formulation than that intended for clinical administration, and considering that the recommended 48 to 96 hour local tolerance studies were not performed, as mentioned in the guideline CHMP/SWP/2145/00 [23], the local tolerance of the final formulation could not be predicted. However, in clinical use, no significant local toxicity was documented, and therefore, the CHMP considered the final formulation non-irritant.

- Other toxicity studies

In vitro photo-cytotoxicity and photo-clastogenicity tests, and a photo-allergenicity investigation that included a preliminary single-dose photo-irritation study were conducted. A summary of photo-safety studies is provided in table 5:

Table 5: Photo-safety studies

Study	System	Route	Duration	Group size	Doses Tested	Report
Photocytotoxicity	Balb/c3T3 cells	*in vitro*	Single dose	6 wells	0.78-100µg/ml	PALO-01-14
Photo-clastogenicity	CH V79 cells	*in vitro*	3 hours	duplicate cultures	125-2000µg/ml	PALO-01-16
Photoirradiation and Photoallergenicity	Guinea pig	dermal	Single dose 2w induction, challenge at 3w	4 females 10 control 20 test females	0, 10, 15, 25, 50 % dilution	PALO-01-15

None of these studies indicated any potential photo-safety concerns.

- Ecotoxicity/environmental risk assessment

The excretion of the medicinal product was considered to be urinary, and the predominant moiety was the parent molecule (palenosetron). A crude predicted environmental concentration ($PEC_{surfacewater}$) of 7 x 10^{-7} µg/l was calculated for the final formulation.

- Discussion on the non-clinical aspects

Pharmacology
Palonosetron hydrochloride is a potent and selective serotonin (5-hydroxytryptamine or 5-HT) receptor antagonist. Palonosetron has shown little or no affinity for other bioreceptors, including other serotonergic receptors.
Palonosetron was able to ameliorate the emetic effects of cisplatin, dacarbazine, actinomycin-D and mechlorethamine in relevant animal models. The anti-emetic effect of palonosetron was more potent and prolonged than that of granisetron and ondansetron.
The primary non-clinical pharmacology studies provided in this dossier satisfactorily addressed the non-clinical pharmacology profile of palonosetron to support the claimed indication.
Concerning cardiovascular safety, *in vitro* studies confirmed the expected effects of palonosetron on I_{Kr} and I_{Na} currents and action potentials, known as class effects of 5-HT_3 antogists, but at very high concentrations (see SPC section 5.3). *In vivo* studies using several species showed effects on cardiac conduction, but no Torsades de Pointes were observed, despite doses of up to 1 mg/kg, which is 300-fold higher than the therapeutic dose in human.
No pharmacodynamic interactions were observed in animal tumor models.

Pharmacokinetics

Pharmacokinetics were investigated by both oral and i.v. routes in the rat, dog and monkey. Although not specifically determined, it is likely that primates are similar to dogs for such studies. Palonosetron was rapidly absorbed, with a low bioavailability, suggesting extensive pre-systemic metabolism. Clear differences were observed in clearance rates and plasma half-life between species, with monkeys showing a slower clearance profile. Humans, at therapeutic doses, demonstrated a linear PK profile with a similar volume of distribution and considerably slower clearance rates and plasma half-life.

Palonosetron was extensively distributed, including to the brain. It did not accumulate and is rapidly cleared. No metabolites were measured in the brain, suggesting that they did not pass the blood-brain barrier or were cleared very rapidly.

Although a range of metabolites was identified from animal oral studies, many were not relevant to the clinical situation, being products of first-pass metabolisms.

Protein binding was approximately 48 % in rat and 66 % in dog plasma. The moderate extent of plasma protein binding suggests that small changes would have no influence on palonosetron availability. The absence of any evidence of enzyme induction and the fact that inhibition was only apparent at high concentrations *in vitro* suggested that interactions mediated by metabolic enzymes were unlikely. One pharmacology experiment on the relationship between plasma concentrations of palonosetron and the protection of dogs against cisplatin-induced emesis (AT 6313) did not suggest any significant difference in kinetics compared with other oral dog studies at similarly low dosages.

Whilst it is acknowledged that every drug combination cannot be practically studied, the CHMP has been concerned about the lack of discussion on possible adverse (synergistic) effects with co-administered medicinal products and the consequences of co-administration of other CYP 2D6 inducers (such as dexamethasone). The applicant was asked to consider the sufficiency of these interactions studies, and to put forward a rationale for the safety of palenosetron in the likely clinical situation of polypharmacy.

Elimination of palonosetron from tissues was parallel to plasma clearance, except in the eye.

As no pharmacokinetic data were provided concerning palonosetron excretion in breast milk, breast-feeding should be discontinued during therapy (see SPC section 4.6). Moreover, the lack of information on placental transfer has been included in the SPC (see SPC section 4.6).

Toxicology

The studies submitted to assess the repeated dose toxicity of palonosetron in mice used the oral route. However, the applicant's justification for performing oral studies to mimic the exposure profile during intravenous administration in humans was considered acceptable.

The CHMP assumed that, with the intended clinical human exposure being a single i.v. injection (the intended clinical i.v. dose of palenosetron (0.25mg) equates to approximately 0.004 mg/kg for a 70 kg adult), and at a dose many multiples lower than the lowest animal i.v NOAEL (7 mg/kg/day and 6 mg/kg/day for rat and dog, respectively), palenosetron, in the absence of drug interactions, was safe for the intended use in human.

According to the results obtained and to the ICH guideline on genotoxicity (CHMP/ICH/141/95)[24], palonosetron was considered as non-genotoxic (see SPC section 5.3).

Two long-term studies assessed the carcinogenic potential of palonosetron in rat and mouse. Although the oral gavage route was used in these studies, whereas bolus intravenous is the route of administration of the proposed indication, all dosages used were multiples of the proposed human dosage and comparison of $AUC_{0\text{-}24h}$ values indicated large multiples, ranging from 136 to 1220-fold in males and from 61 to 706-fold in females. High doses applied daily for two years caused an increased rate of liver tumours, endocrine neoplasms (in thyroid, pituitary, pancreas, adrenal medulla) and skin tumours in rats but not in mice. The underlying mechanisms are not fully understood, but because of the high doses employed and since palonosetron is intended for single application in humans, these findings are not considered relevant for clinical use (see SPC section 5.3).

The reproductive and developmental studies conducted were appropriate and the NOAELs were high enough to allow a reasonable assumption of safety in human.

No evidence of local tolerance toxicity was observed in the i.v. toxicology investigations.

Two impurities, the final intermediate 07-PALO and the diastereoisomer 09-PALO, have been qualified by their presence in all batches of active substance used for the pivotal studies throughout toxicity testing. It was considered that the presence of 07-PALO at not more than 1% in the medicinal

product was not at risk for the use of palonosetron in clinical use. The lack of 09-PALO toxicity studies was appropriately justified.
From other toxicity studies, two impurities, the final intermediate 07-PALO and the diastereoisomer 09-PALO, have been qualified by their presence in all batches of active substance used for the pivotal studies throughout toxicity testing and the NOAEL was established. These impurities were considered unlikely to represent a human hazard.
In the environmental risk assessment showed that the PEC was well below the action value of 0.01µg/l, as defined in the CHMP on environmental risk assessment of medicinal products for human use and therefore, no environmental effects are therefore anticipated from the use of palonosetron [25].

4 Clinical aspects

Introduction

All trials were conducted according to the principles of GCP.
A total of 18 clinical studies have been submitted. These studies were carried out in patients in healthy volunteers and special populations using i.v. or oral administration.
Studies performed with intravenous palonosetron in CINV are shown in table 6.

Four clinical studies (2330, PALO-99-03, PALO-99-04, PALO-99-05) investigated the efficacy of an intravenous single dose of palonosetron during the acute period (24 hours), in adult patients receiving either moderately or highly emetogenic chemotherapy. Three studies were pivotal (PALO-99-03, PALO-99-04, PALO-99-05). Patients having completed one of the three studies could be enrolled in study PALO-99-06, an uncontrolled study that investigated the efficacy of repeated doses of palonosetron i.v. in consecutive chemotherapy cycles.
An additional phase II study in CINV (study 2120) was discontinued after only two patients were enrolled due to the slow enrolment rate and did not contribute to the efficacy data.
A paediatric study in patients with CINV was ongoing at the time of the submission of the application.

Table 6: Summary of phase II and III trials performed with intravenous palonosetron

Study Number	Study Population	Dosage Range	Comparator (dose)	Number of patients
2330 / PALO-00-01	Patients with highly emetogenic CINV	.3, 1, 3, 10, 30, and 90 µg/kg single i.v. dose < .1, .25, .75, 2, or 6 mg single i.v. dose	2330 Not applicable PALO-00-01 historical placebo	161 randomised
PALO-99-03	Patients with moderately emetogenic CINV	.25 or .75 mg single i.v. dose	Ondansetron 32 mg i.v.	570 randomised
PALO-99-04	Patients with moderately emetogenic CINV	.25 or .75 mg single i.v. dose	Dolasetron 100 mg i.v.	592 randomised
PALO-99-05	Patients with highly emetogenic CINV	.25 or .75 mg single i.v. dose	Ondansetron 32 mg i.v.	680 randomised
PALO-99-06	Patients with moderately or highly emetogenic CINV, recruited in studies PALO-99-03, -04 and -05	Repeated .75 mg single i.v. doses	Not applicable	905 enrolled (1733 chemotherapy cycles)

Abbreviations: [a] : Original protocol called for dose range of 0.3 to 30 µg/kg, but it was amended to drop the 0.3 µg/kg dose and add a 90 µg/kg; CINV: Chemotherapy induced nausea and vomiting

Pharmacokinetics

The pharmacokinetics and dose proportionality of palonosetron following intravenous and orally administered have been conducted in healthy volunteers (studies 2092, 0100, 2236 and 0101), in CINV patients (studies 2120, 2330 and 2332) and in PONV patients (studies 2500 and 2502).
An ADME trial with radiolabelled palonosetron in healthy volunteers was performed (study 2216).
Three special population studies were conducted in patients with renal (study PALO-99-35), hepatic impairment (study PALO-99-51), and patients who were poor or extensive metabolisers of cytochrome P450 2D6 (study PALO-99-39).
A pharmacokinetic interaction study between palonosetron and metoclopramide (study PALO-99-34) was performed.
A population pharmacokinetic analysis (study PALO-99-33) on the concentration data derived from a large subset of patients enrolled in main studies (studies PALO-99-03, PALO-99-04, and PALO-99–05) was performed.

- Method

Palonosetron and M9 (primary metabolite) plasma and urine concentrations were evaluated using HPLC-RIA, LC/MS and LC/MS/MS analytical methods.

- Absorption

Healthy volunteers
Following single i.v. doses administered either as a five-minute infusion (study 2092, N = 60) or as a 30- second bolus dose (study 0100, N = 24), plasma concentrations of palonosetron initially declined rapidly. Following this decline, notable increases or secondary peaks were seen in some subjects at approximately two to four hours post-dosing.
Following oral dosing (Studies 2236, N = 29 and 0101, N = 24), mean palonosetron C_{max} (0.348 to 14.1 ng/ml) was achieved between three and six hours after doses ranging from 3.0 µg/kg to 90 µg/kg in healthy subjects. The plasma C_{max} and AUC values of palonosetron increased with dose, but the dose proportionality over the doses tested could only be demonstrated in one study.

Patients
In patients with CINV (studies 2330, 2332 and 2120) and patients with PONV (studies 2500 and 2502), palonosetron doses ranged from 0.1 to 90 µg/kg administered either intravenously (studies 2330, 2120 and 2500) or orally (studies 2332 and 2502).
Plasma concentrations of palonosetron increased proportionally after i.v. bolus injection in both CINV and PONV populations. T_{max} after i.v. administration was within the first sampling times for most patients, although there were individual patients with T_{max} values occurring later in the concentration *versus* time profile (see table 7).
The next table summarises pharmacokinetic results for i.v. palonosetron Phase II studies. The 3 µg/kg dose is approximately equivalent to the 0.25 mg fixed dose, which is the sole dose requested for approval in this application.

Following oral dosing in patients, plasma concentrations increased proportionally, as measured by C_{max} and AUC, with a mean T_{max} generally occurring between 4 and 6 hours over the dose range tested.

Bioavailability
No bioavailability studies were submitted.

Influence of food
No food effect studies were submitted.

- Distribution

Healthy volunteers
The mean volume of distribution of palonosetron in healthy volunteers (studies 2092, 0100, 2236, 0101), following oral or i.v administration, ranged between 3.85 l/kg and 12.6 l/kg.

Patients
The mean volume of distribution of palonosetron in patients (studies 2330, 2500), following i.v administration, ranged between 5.96 and 12.5 l/kg.

Population Pharmacokinetics
Palonosetron disposition was characterised by a two compartment open model with a median systemic clearance estimated at 3.25 l/h and a large volume of the central compartment, estimated to be 632 l. The intercompartment clearance and volume of the peripheral compartment were estimated to be 4.91 l/h and 1740 l, respectively, indicating extensive tissue distribution. Although interindividual variability in clearance was large (88.8%), none of the covariates investigated were predictive of palonosetron clearance. No effect of age, gender, race, co-medications, or clinical chemistries on palonosetron clearance was observed. Volume of distribution was associated with body weight and negatively associated with serum albumin concentrations and Karnofsky performance status, although, together all the covariates only explained 12% of the interindividual variability in volume of distribution.

Protein Binding
Binding in human plasma was constant over the concentration range evaluated (5 to 412 ng/ml) and averaged approximately 62%. An *in vitro* study demonstrated non-specific and non-saturable binding of palonosetron to plasma proteins.

- Elimination

Metabolism
The biotransformation of palonosetron has been characterised based on *in vitro* studies (PALO-98-02: CYP450 mediated metabolism of palonosetron; PALO-00-02: identification of the human metabolite M4), and clinical studies (Study 2216: ADME; study PALO-99-39: CYP2D6 polymorphic metabolism)

Approximately 50% of palonosetron was metabolised by the liver, almost exclusively by the CYP450 through N-oxidation and monohydroxylation. Oxidation at the nitrogen formed the N-oxide (M9) and hydroxylation formed the 6-S-hydroxy-palonosetron (M4). These metabolites had low 5-HT_3 antagonist activity, approximately 1% of that of the parent compound. Keto-palonosetron (M6) was a minor component found in urine and plasma whereas trace amounts of 6-keto-palonosetron (M5) were detected only in plasma.

Oxidation to M9 was dependent on CYP2D6 with contributions from CYP3A4 and CYP1A2. Neither palonosetron nor M9 induced cytochrome P450. At high concentrations *in vitro*, palonosetron was a competitive inhibitor of some cytochrome isoforms but this was not considered to be clinically relevant.
In studies 2092 and 0100 (i.v. administration in healthy volunteers), M9 was not measurable in plasma at doses of palonosetron below 10 µg/kg, and in many cases the plasma concentrations at the 10 µg/kg dose level were not quantifiable. Mean M9 C_{max} was similar in both studies (ranging from 0.093 to 0.777 ng/ml), but T_{max} generally ranged from approximately 3 to 6 hours. Mean half-life was 19.3 to 54.3 hours in study 2092 and 24.3 to 37.4 hours in study 0100.
As with the intravenous route of administration, the proportion of M9 in plasma was small compared to palonosetron. The lower range of T_{max} was approximately 2 hours in both studies, while the upper range varied between 3 hours and over 44 hours. Mean half-life ranged from 25.8 to 35.3 hours. Mean AUC ratios of M9 and palonosetron indicated that a small amount of metabolite was present in plasma relative to the parent compound. The percentage of administered palonosetron excreted as M9 generally decreased with increasing dose levels.
Considering the prolonged half-lives in a subset of patients, study PALO-99-39 was designed to investigate (1) the influence of the metaboliser status on the PK profile of palonosetron and M9, and (2) the presence in human plasma and urine of RS-42358 (the last intermediate in palonosetron

synthesis) and of its N-oxide metabolite RS-72033. This study indicated that there was no effect of extensive or poor metaboliser status on the pharmacokinetics of palonosetron and M9. Nor RS-42358 or RS-72033 were detected in plasma or urine after single i.v. dosing of palonosetron 0.75mg.

Excretion

Approximately 40% of palonosetron is renally cleared unchanged.
Following i.v. administration in healthy volunteers (study 2216, N = 6), palonosetron was eliminated from the body with an apparent mean plasma elimination half-life of 37.4 hours (mean range 30.8 to 54.1 hours). The total body clearance of palonosetron was 173 ± 73 ml/min and renal clearance was 53 ± 29 ml/min (studies 2092 and 0100).
In patients studies, mean i.v. clearance generally ranged between 1.51 ml/min/kg and 2.23 ml/min/kg and did not change with dose except in the low-dose groups (study 2500), where clearance was estimated as less than 1 ml/min/kg (see table 7). Mean half life ranged from 38 to 128 hours. Some patients in each trial demonstrated much longer half-lives, in all 114 subjects who provided PK data from Phase II trials, 11 exhibited half-lives of greater than 100 hours (ranged from 108 hours to 383 hours).
ADME study (2216) investigated the routes and rates of excretion, the pharmacokinetics of total radioactivity, and the metabolic profile in urine and plasma following a single intravenous dose of 10 µg/kg [^{14}C]-palonosetron to healthy volunteers. Palonosetron represented the highest percentage of radioactivity in plasma (approximately 72 % in the 0 to 96 hour interval). The mean terminal elimination half-life of palonosetron was 37.4 hours. Transient and very low concentrations of metabolites M9, M5 and M4 were also detected in plasma, but concentrations were too low to estimate pharmacokinetic parameters for these metabolites.
Excretion in urine was almost complete over the 0 to 144 hour interval, and 83.2 % of the administered dose was recovered in urine (over 0- 240 hours). Three major radioactive components were detected in urine: palonosetron (39.3 % of the administered dose), M9 (12.5 %) and M4 (10.9 %). In faeces, recovery was 3.38 %.

- Dose proportionality and time dependencies

In patients, plasma concentrations of palonosetron increased proportionally after intravenous bolus injection or after oral administration in both CINV and PONV populations (studies 2330, 2332; 2500, 2502). Similar results were observed in healthy volunteers.

Table 7: Mean pharmacokinetic parameters of palonosetron after a single i.v. dose of 0.1 to 90 µg/kg

Study 2330 (n=37, CINV)						
Dose [a] (µg/kg)	**C_{max} (ng/ml)**	**T_{max} (hr)**	**$t_{1/2}$ hr)**	**Total AUC (ng·hr/ml)**	**CL (ml/min/kg)**	**V_d (l/kg)**
1 (n=6)	0.88	0.150	128 [c]	13.8[c]	1.51 [c]	12.5 [c]
3 (n=6)[b]	5.63 [b]	0.144	56.4 [c]	35.8 [c]	1.66 [c]	7.91[c]
10 (n=5)	13.0 [b]	0.827	49.8	81.8	2.23	9.56
30 (n=8)	35.7	0.360	86.4	348	2.13	9.18
90 (n=12)	336	0.564	43.7	957	1.90	6.83
Study 2500 (n=29, PONV)						
0.1 (n=4)	0.923	0.113	38.0	5.23	0.320	1.07
0.3 (n=7) [d]	4.28	0.0452	91.7	23.6	0.659	5.96
1 (n=5)	1.87	0.0333	110	21.3	1.07	6.68
3 (n=6)	7.89	0.0306	51.9	28.4	1.83	8.20
30 (n=7)	29.6	0.0190	53.7	302	2.14	10.0

Abbreviations: [a]:3 µg/kg dose is approximately equivalent to the 0.25 mg fixed dose; [b]: Mean ± SD for 3 µg/kg and 10 µg/kg dose groups are 5.63 ± 5.48 ng/ml and 13.0 ± 20.1 ng/ml, respectively; [c]: n = 5; [d] Mean value is skewed by a single patient with extremely high concentrations; AUC = area under the concentration versus time profile; C_{max} = maximum concentration in a plasma concentration versus time profile; CL = clearance, $t_{1/2}$ = elimination half-life, T_{max}= time of maximum concentration in a plasma concentration versus time profile, Vd = volume of distribution.

Repeat doses data were provided from a phase I (PALO-02-12) and a phase II (2330: see dose response study description and table 7) clinical trial, as well as from pharmacokinetic simulations based on data derived from these two studies.

Study PALO-02-12

This was a repeated-dose, double-blind study in which subjects were randomly assigned to receive intravenous bolus of palonosetron 0.25 mg or placebo intravenously, once daily, on 3 consecutive days (N = 12 and 4, respectively).

Plasma drug concentrations declined in a biphasic manner, with a rapid initial distribution phase followed by a slower elimination phase. Palonosetron was measurable in the plasma 168 hours after the third administration. The mean $t_{1/2}$ was 42.8 hours. A 2.1-fold accumulation of drug in plasma occurred after 3 daily administrations (accumulation ratio of day 3 to day 1 $AUC_{(0–24)}$ values), which was consistent with the long plasma elimination half-life.

Pharmacokinetic Simulations

- Three consecutive daily doses:

This simulation included an extreme case with $t_{1/2}$ of 350 hours receiving 3 daily doses (representing an average half-life between the two longest observations seen in Phase I [309 hours] and Phase II [383 hours] studies), simulations for two data sets.

For the extreme case scenarios in subjects with a terminal half-life of 350 hours receiving three consecutive daily doses, the $AUC_{0-\infty}$ values were 596 µg·h/L and 806 µg·h/L. These values were lower than the mean value (957 µg·h/L) obtained after one single 90 µg/kg dose in study 2330.

The mean total $AUC_{0-\infty}$ for the 3 days in healthy volunteers participating in PALO-02-12 was estimated at 77 µg·h/L (mean half-life 42 hours). This AUC value was approximately 8-fold lower than the lowest simulated value and 11-fold lower than the highest simulated value in a theoretical subject with a 350-hour half-life.

The $AUC_{0-\infty}$ estimations in the extreme case scenarios were obtained over the dosing interval plus the time of 4 half-life values and extrapolated to infinity. The total time span for exposure was thus more than 60 days. For the highest simulated $AUC_{0-\infty}$ value, this equals an estimated AUC per each 24 hours exposure of approximately 13 µg·h/L, which is lower than the estimated daily exposure in study PALO-02-12.

C_{max} values for the simulated extreme case (2697 ng/L and 2983 ng/L) are within the range of those observed for subjects on day 3 in study PALO-02-12 (mean C_{max} value; 2430 ng/L, range 671 ng/L – 3780 ng/L).

- Multiple cycle exposure (once weekly):
This simulation included multiple cycle chemotherapy with weekly intervals for 5 consecutive weeks (upper end of the amount of cycles expected) using both the extreme case ($t_{1/2}$ = 350 hours) and the expected case ($t_{1/2}$ = 40 hours), simulations for two data sets each.
For the extreme cases (half-life of 350 hours), the total drug exposure ($AUC_{0-\infty}$) was calculated as 993 µg·h/L and 1344 µg·h/L for each representative subject. These values were lower than the highest observed systemic exposure in one patient after the administration of 90 µg/kg (i.e. 1721 µg·h/L) whose half-life was 70 hours (study 2330). The adverse event profile for the subject with the highest exposure, however, was unremarkable. The 5 consecutive week dosing schedule plus the washout time results in approximately 12 weeks (87 days) of exposure for the most extreme cases, and exposure based on a per day calculation which are less than that observed in study PALO-02-12.
In the same model, for a subject with a 40-hour half-life, the simulated total drug exposure was 146 µg·h/L and 187 µg·h/L for the two representative profiles. This exposure was likely to occur in approximately 90% of the patients and it is about 5-7-fold lower than that observed for the extreme long half-live cases.

- Special populations

Impaired renal function

The influence of renal impairment was studied in subjects with mild to severe renal impairment (PALO-99-35). This study was a Phase I, open-label, single dose, parallel group study to evaluate the pharmacokinetics of palonosetron and M9 in subjects (N = 25) with mild to moderate (Clcr 30-80 ml/min; N = 9) and severe (Clcr 10-29 ml/min; N = 7) renal impairment (RI) after receiving a single intravenous dose of 0.75 mg administered as bolus over 30 seconds. The reference group consisted of healthy volunteers (N = 9), with a creatinine clearance of > 80 ml/min.

The mean $AUC_{0-\infty}$ for healthy subjects (83.8 h·µg/L) was comparable to those subjects with mild (88.8 h·µg/L) or moderate (80.9 h·µg/L) renal failure. Subjects with severe renal failure had a mean systemic exposure of 133.6 h·µg/L (range of 42.2 to 228.9 h·µg/L).

Impaired liver function
A Phase I, open-label, single dose, parallel group study was conducted in 24 patients to assess the effects of varying degrees of hepatic impairment (8 patients per Child-Pugh group) on the pharmacokinetics of palonosetron and M9 in comparison to healthy subjects (PALO-99-51). Patients received a single i.v. dose of 0.75 mg palonosetron. The control group consisted of 9 healthy subjects treated in study PALO-99-35 (PK in renal impairment).
Hepatic impairment was associated with significantly lower palonosetron C_{max} values, particularly in subjects with severe impairment. Plasma concentration curves in subjects with hepatic impairment were similar to those in healthy subjects, but several curves were notably different. Instead of an initial decline in the plasma concentrations, an initial rise was seen from 15 minutes onward, with C_{max} being reached between 0.5 and 2 hours after i.v. dosing. Secondary peaks after an initial rapid decline were seen in several healthy subjects, generally limited to a slight increase in the plasma concentration followed by a further decline. The terminal elimination half-life tended to be longer in the patient with moderate and severe hepatic impairment, also the distribution volume was clearly higher in severe hepatic impairment group.
Palonosetron $AUC_{0-\infty}$ and total body clearance values were similar in impaired and healthy subjects.
For the M9 metabolite, the mean C_{max} and $AUC_{0\text{-tlast}}$ values were decreased with moderate and severe hepatic impairment.
Excretion of palonosetron was similar in all groups. The excretion of M9 was slightly higher in the healthy volunteers than in patients with hepatic impairment, although no clear relation between the M9 excretion and degree of hepatic impairment was apparent. Excretion of M9 continued for several days after the time of the last measurable plasma concentration, and was complete at 240 hours in approximately one third of the study participants. The sum of palonosetron and M9 recoveries ranged between 37 % and 50 % of the dose.

Gender
In the population pharmacokinetic study, no effect of gender on palonosetron clearance was observed.

Race
No formal pharmacokinetic study was conducted in different ethnic groups. Data were gained from the population pharmacokinetic analysis where it was shown that pharmacokinetic parameters were similar in subjects of Caucasian and Hispanic origin. However, the volume of the central compartment was increased by 20% in Caucasians relative to other races. Race had no effect on clearance. No conclusion on PK in Black or Asian races could be made due to the limited number of patients.

Weight
Volume of distribution was correlated with weight in the population PK/PD analyses. However, overall this did not account for much of the inter-individual variability in clearance which was large at 88.8%.

Elderly
No formal pharmacokinetic study was conducted in the elderly. In the population pharmacokinetic analyses, pharmacokinetic parameters were similar in subjects aged 65 years or more (65 to 91 years) and younger subjects (18 to 64 years).

Children
No formal pharmacokinetic study was conducted in the children. A Phase III trial (study PALO-99-07) to assess the safety, pharmacokinetics and efficacy of single i.v. 3 or 10 µg/kg dose of palonosetron in paediatric patients receiving moderately or highly emetogenic chemotherapy was ongoing at the time of the assessment of this MAA. The planned number of subjects was 72. The completion of this study was foreseen during year 2005.

- Pharmacokinetic interaction studies

In vitro
Incubations of palonosetron with pooled human liver microsomes in the presence and absence of palonosetron showed that palonosetron was a competitive inhibitor of the same three isozymes involved in its metabolism (CYP2D6, CYP3A4 and CYP1A2). However, inhibition constants (K_i) indicated that palonosetron has no inhibitory potential at clinically relevant concentrations. Lastly, induction experiments with fresh human hepatocytes demonstrated that palonosetron did not induce cytochrome P450 isozymes at clinically relevant concentrations.

In vivo
A study (PALO-99-34; N = 11) has been conducted to evaluate potential pharmacokinetic interactions between a single i.v. dose of palonosetron and steady state concantration of oral metoclopramide. No differences were observed in palonosetron or M9 plasma concentrations with or without metoclopramide.
In controlled clinical trials, palonosetron has been administered with corticosteroids, analgesics, antiemetics, antinauseants, antispasmodics and anticholinergic agents.

Pharmacodynamics

- Mechanism of action

No clinical pharmacodynamic have been submitted.

- Primary pharmacology

No primary pharmacology have been submitted.

- Secondary pharmacology

Cardiovacular pharmacology
Evaluation of ECG tracings from phase I and II clinical trials have been submitted.
A confirmatory population pharmacodynamic evaluation was performed in phase III trials. Patients received either palonosetron 0.25 mg (N = 605) or 0.75 mg (N = 610), ondansetron 32 mg (N = 410), or dolasetron 100mg (N = 194). The integrated analysis, the effect on the QTc parameter by Bazett or Fredericia correction was 2 msec at both palonosetron doses. No patients had more 60 msec change from baseline.
An integration of selected phase I, II and III trials (studies 2236, 0101, 2216, 2092, 0100, 2332, 2330, PALO-99-03, PALO-99-04, PALO-99-05) was performed. The results of QT/QTc are shown in table 8.

Table 8: QT/QTc from phase I, II and III trials (mean changes from baseline)

	Palonosetron doses				**Active comparator**	
	Palonosetron < 0.25 mg	**Palonosetron 0.25 mg**	**Palonosetron 0.75 mg**	**Palonosetron > 0.75 mg**	**Ondansetron 32 mg**	**Dolasetron 100 mg**
N	59	667	678	181	404	192
QT, msec	-1	3	3	1	5	6
QTcB, msec	4	1	2	2	4	5
QTcF, msec	2	2	3	2	5	5

Abbreviations: msec = milliseconds; N = number of subjects with data at least one tracing;
QTcF = QT interval corrected by Fridericia formula; QTcB = QT interval corrected by Bazett formula

The mean change identified was about 2–3 msec (QTcF). The active controls demonstrate a consistent QTcF change from baseline of 5 msec, which was about two-fold greater than the 2.5 msec change on palonosetron.
Holter monitoring was collected in a subset of subjects at selected centres in the pivotal phase 3 trials by which an attempt was made to determine if any arrhythmias in the therapeutic arms could be identified. The evaluable Holter data (143 patients on palonosetron and 50 patients on ondansetron or dolasetron) did not provide any signal that palonosetron induces clinically relevant supraventricular or ventricular arrhythmias, including Torsades des Pointes, or atrio-ventricular conduction defects.

Gastrointestinal pharmacology
No clinical studies have specifically investigated the effect of palonosetron on the gastrointestinal tract.

Discussion on Pharmacokinetics / Pharmacodynamics

Plasma kinetics in man following intravenous treatment were determined using similar methods to those used in animals treated intravenously. The data obtained differed significantly from those in any of the animal species, principally due to a much slower clearance and a considerably greater half-life with a consequently disproportionately higher AUC.

The clinical pharmacokinetic profile of palonosetron was characterised by a rapid initial decline of plasma concentration following single intravenous doses, with secondary peaks observed in some subjects approximately two to four hours after dosing. This profile was consistent with a possible entero-hepatic recycling. The applicant has not specifically evaluated these mechanisms either in animal models or in humans, since this was not expected to contribute to the overall exposure or to be clinically relevant.
After single intravenous dosing, AUC and C_{max} increased almost dose proportional over the dose range of 0.3 to 90 µg/kg. Palonosetron has a half-life considerably longer than that of other 5-HT_3 antagonists (approximately 40 hours compared to 3-12 hours). Furthermore, in some patients (about 10%), the half-life was exceptionally long (up to 300 hours). The wide inter-individual variability in clearance and half-lives was not considered to have an impact on clinical efficacy and safety.

Results from studies PALO-02-12, 2330 and from a number of PK simulations, accumulation of palonosetron occurred with daily doses of 0.25 mg. While high exposure was shown to be safe and well tolerated, the SPC has been amended to advise against repeat dosing within 7 days.
The main metabolites M9 and M4 had low 5-HT_3 antagonist activity (approximately 1% of that of the parent compound) and therefore were considered to be clinically inactive.

Hepatic impairment did not significantly affect total body clearance of palonosetron compared to healthy subjects. There was a slight trend towards higher AUC with the highest Child-Pugh scores, however, marked variability was observed within each sub-category of the subjects with hepatic impairment. Therefore, while the terminal elimination half-life and mean systemic exposure of palonosetron was increased in subjects with severe hepatic impairment, no dose adjustment is necessary for patients with impaired hepatic function (see SPC section 5.2).

No significant effect on palonosetron clearance when co-administered with CYP2D6 inducers (dexametasone and rifampicine) and inhibitors (including amiodarone, celecoxib, chlorpromazine, cimetidine, doxorubicine, fluoxetine, haloperidol, paroxetine, quinidine, ranitidine, ritonavir, sertraline or terbinafine) was shown (see SPC section 4.5).

Approximately 40% of palonosetron was renally cleared unchanged. In the faeces recovery was around 3% of the dose over the interval 0 to 144 hours.

Age, gender, weight, race had no clinically relevant influence on the pharmacokinetics of palonosetron according to pharmacokinetic population study. No formal pharmacokinetic study was conducted in elderly or in children (see SPC section 5.2). No dosage adjustment is necessary for the elderly (see SPC section 4.2).

AUC *versus* creatinine clearance as a continuous variable showed, as would be predicted from the high volume of distribution of palonosetron, no obvious influence of reduced renal function on the systemic exposure of palonosetron following an i.v. dose. Although mean $AUC_{0-\infty}$ appeared higher in subjects with severe renal impairment, no dose reduction was considered necessary in renal insufficiency patients (see SPC section 4.2).
No studies have been performed in patients with end stage renal disease requiring dialysis (see SPC section 4.2). However, due to the large volume of distribution and relatively equal contribution of hepatic metabolism and renal elimination of palonosetron, dialysis is not likely to affect the clearance of palonosetron, and therefore is unlikely to be an effective treatment for palonosetron overdosage (see SPC section 4.9).
No pharmacokinetic data were available in paediatric patients at the time of the assessment. The use of palonosetron in patients under 18 years of age is not recommended until further data become available (see SPC section 4.2 and post-approval commitments on study PALO-99-07).
There is no experience of palonosetron in human pregnancy so palonosetron should not be used in pregnant women unless it is considered essential by the physician (see SPC section 4.6).

The interaction potential of palonosetron has been appropriately studied *in vitro*. At high concentrations *in vitro*, palonosetron was a competitive inhibitor of some cytochrome isoforms (CYP2D6, 1A2 and 3A). Since *in vivo* concentrations of palonosetron were much lower, the inhibition potential of palonosetron has no clinical implications regarding cytochrome P450. *In vitro*, palonosetron was not shown to be an inducer of the activity of CYP2D6, CYP1A2, or CYP3A4/5.

No significant pharmacokinetic interaction was shown between a single intravenous dose of palonosetron and steady state oral metoclopramide. Concomitant administration of medicinal products known to inhibit the primary mechanisms of hepatic elimination (including cimetidine and haloperidol) is not expected to alter the pharmacokinetic parameters of palonosetron. Lorazepam is metabolized almost entirely by conjugation and a significant interaction is considered unlikely, as it has no significant effects on the metabolic pathway of palonosetron.

Like other 5-HT_3 antagonists, palonosetron possesses the ability to block ion channels involved in ventricular de- and re-polarisation and to prolong action potential duration [19, 26-29]. To address the

safety concerns clinically relevant to the QTc prolongation derived from a few preclinical data, the results of the evaluation of ECG tracings from Phase I and II clinical trials have been submitted. No evidence of any cardiac safety risk was observed.
Constipation is a typical adverse event of 5-HT_3 receptor antagonists. However, no clinical studies have investigated the effect of palonosetron on the gastrointestinal tract. The SPC has been amended to address this issue, based on the non-clinical findings with palonosetron and other drugs of this class, and based on the frequency of constipation, as an adverse effect in clinical studies.

Relationship between plasma concentration and effect

The 0.25 mg palonosetron dose represented the lowest dose tested to reach the efficacy plateau, well known for this class of agent [30, 31] and it was considered adequate throughout the 120-hour period.
Additional pharmacodynamic studies were not needed given the non-clinical and clinical experience.

Clinical efficacy

The demonstration of clinical efficacy relied on three main clinical trials (studies PALO-99-03, PALO-99-04, PALO-99-05), and supportive data from Study 2330 (study PALO-00-01) and a trial of palonosetron after repeated cycles (study PALO-99-06).

- **Dose response studies**

Study 2330: Study 2330 was a phase II randomized, double blind, multicenter, dose-ranging efficacy, safety and pharmacokinetic study that used the i.v. route for palonosetron in the patients with CINV.
The study involved 23 centres in the United States and a total of 161 patients were enrolled. One-hundred and forty-eight patients were eligible for the primary analysis of efficacy.
The primary efficacy endpoint was complete response (CR), defined as no emetic episodes and no rescue medication during the first 24 hours after administration of chemotherapy.
The original protocol called for patients to receive one of the following doses: 0.3, 1, 3, 10, and 30 μg/kg. Due to the lack of efficacy, the protocol was firstly amended to discontinue the lowest dose and secondly to add a higher dose (90 microg/kg.). The randomisation code was not broken. No adjustment of the sample was performed. The results relevant to the lowest dose group 0.3 μg/kg were combined with those of the 1 μg/kg group. Response was 24 %, 46%, 40%, 50% and 46% for the 0.3-1, 3, 10, 30 and 90 μg/kg dose groups respectively.
An analysis of the efficacy data obtained from study 2330 compared to a historical placebo control showed that palonosetron doses of 3 to 90 μg/kg, as well as the corresponding converted doses of 0.25 to 6 mg, were significantly superior to the historical placebo group.

- **Main studies**

PALO-99-03

PALO-99-03 was a three-arm double-blind multicentre randomised active-controlled clinical trial of single i.v. doses of palonosetron 0.25 mg or 0.75 mg, *vs.* ondansetron 32 mg i.v., for the prevention of moderately emetogenic CINV.

METHODS

Study Participants

The main inclusion criteria were: age ≥ 18 years old with histologically and/or cytologically confirmed malignant disease, Karnofsky PS ≥ 50 %, scheduled to receive at least one of the following agents or regimens on day 1: carboplatin, epirubicin, idarubicin, ifosfamide, irinotecan, mitoxantrone, methotrexate >250 mg/m^2, cyclophosphamide <1500 mg/m^2, doxorubicin >25 mg/m^2, or cisplatin ≤ 50 mg/m^2 (over 1 to 4 hours). The administration of the main emetogenic agent following the classification probodsed by Hesketh *et al*. [32] on study day 1 should not extend beyond 4 hours. The main criteria for exclusion were use of investigational drugs within 30 days, use of any drug with potential antiemetic efficacy within 24 hours of the start of treatment or scheduled to receive any such drug until day 5 of the study, experience of NCI CTC grade 2 or 3 nausea within

24 hours preceding chemotherapy, ongoing vomiting from any organic etiology, experience of nausea (moderate to severe) or vomiting following any previous chemotherapy. Patients who were scheduled to receive any dose of nitrogen mustards, dacarbazine or streptozocine, lomustine >60 mg/m^2, carmustine >250 mg/m^2 or any other chemotherapeutic agent with an emetogenicity level 5 were not eligible. Also, patients scheduled to receive any chemotherapeutic agent with an emetogenicity level 3 or higher, or scheduled to receive radiotherapy of the upper abdomen or cranium, during days 2 to 6 were not eligible. Only patients having provided a signed written informed consent were eligible.

Treatments
Patients randomised to palonosetron 0.25 mg, or 0.75 mg were administered palonosteron intravenously as a 30 second bolus, followed by a 15 minutes infusion of 0.9% saline. Patients randomised to receive ondansetron were administered 0.9% saline intravenously as a 30 second bolus, followed by ondansetron 32 mg administered intravenously over 15 minutes. Rescue medication for the treatment of nausea and vomiting after chemotherapy, with the exception of study drugs, was permitted at the investigator's discretion. The study drug was administered 30 minutes prior to chemotherapy.

Objectives
The primary objective was to compare the efficacy of single-doses of palonosetron 0.25 or 0.75 mg, to ondansetron 32 mg i.v. in preventing moderately emetogenic CINV. The secondary objectives were to evaluate the safety and tolerability of palonosetron, and the effect of anti-emetic control on quality of life.

Outcomes / endpoints
The primary efficacy endpoint was complete response (CR) during the first 24 hours after administration of chemotherapy. CR was defined as no emetic episodes and no rescue medication. An emetic episode was defined as one occurrence of vomiting or a sequence of occurrences in very close succession not relieved by a period of relaxation, any number of occurrences of unproductive emesis (retches) in a unique 5-minute period, or an episode of retching of less than 5 minutes duration combined with vomiting not relieved by a period of relaxation. Relaxation between episodes was defined as a period of at least 1 minute. Diaries were used to record emetic episodes, use of rescue medication and severity of nausea, at 24-hour intervals for up to 120 hours.
Secondary endpoints were the proportion of patients considered to have achieved a CR during the 24 to 120 hour time period (day 2 to 5), during the 0 to 48, 0 to 72, 0 to 96 and 0 to 120 hour period of time (days 1 to 2, 1 to 3, 1 to 4 and 1 to 5, respectively) and during the 24 to 48, 48 to 72, 72 to 96 and 96 to 120 hour time period (days 2, 3, 4 and 5 respectively); the proportion of patients considered to have achieved complete control (CC), defined as no emetic episode, no rescue medication and only mild or no nausea daily for the 0 to 120 hours interval and for the 0 to 48, 0 to 72 and 0 to 96 and 0 to 120 hours time period; time to treatment failure (based on time to first emetic episode or time to rescue medication, whichever occurred first); the number of emetic episodes daily for the 0-120 hour interval and for the overall 0-120 hour interval (study day 1 to 5); the time to first emetic episode; time to administration and need for rescue medication. Severity of nausea was measured by patients using the 4-point Likert scale (none, mild, moderate, severe), daily for the 0 to 120 hour interval. Patient global satisfaction with anti-emetic therapy was measured using a visual analogue scale (VAS, from 0 “not at all satisfied” to 100 “totally satisfied”), daily for the 0 to 120 hour interval. Quality of life was measured using a modified [33] Functional Living Index-Emesis (FLIE) questionnaire for the first 24 hour time period and the standard FLIE for the 24 to 96 hour time period [34].
Sample size
A sample size of approximately 567 patients (189 patients per arm) was calculated based on an assumed response rate of 70%, a difference of no more than 15% in CR proportion, and setting α=0.0125 (*i.e.* α=0.025/2 to adjust for the two treatment comparisons) for a one-sided test of equivalence, 80% power for each comparison, and a 5% dropout.

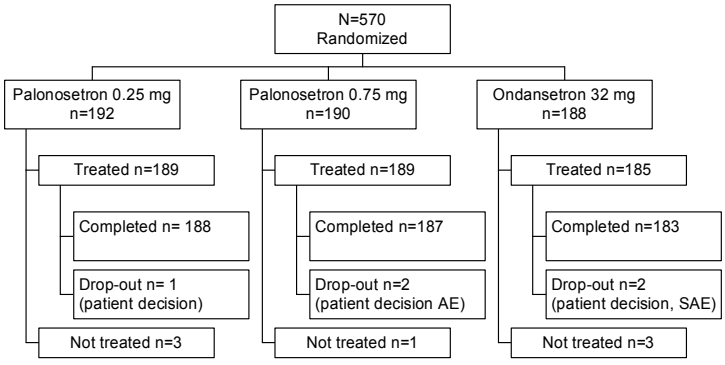
Randomisation
Randomization (as amended) was stratified by gender, previous chemotherapy history (naive *v.* non-naive), using an adaptive dynamic method (minimisation). Use of i.v. dexamethasone was added through an amendment as a stratification factor at randomization but the amendment only came into effect after recruitment had been completed.

Blinding (masking)
The trial was double-blind.

Statistical methods
The trial was designed to show non-inferiority of at least one dose of palonosetron to active comparator with respect to the primary efficacy endpoint. The non-inferiority margin was chosen based on a literature based meta-analysis of trials with approved antiemetics or placebo. The meta-analysis was also used to identify important prognostic factors, and to provide a historical comparison with placebo for validating the effect observed for the active control arm (data not shown).
To test this hypothesis, the lower bound of a two-sided 97.5% confidence interval for the difference in proportions was used. The confidence interval was calculated using approximate methods [33]. Concerning secondary endpoints, differences in the number of emetic episodes, severity of nausea, patient global satisfaction, and quality of life were explored using standard non-parametric techniques (Kruskal-Wallis and Wilcoxon tests). Differences in time to first emetic episode were explored using the log-rank test. Additional exploratory analyses were conducted using standard methodology (subgroup analysis, logistic regression).
The population for the primary efficacy analysis (so-called "intention-to-treat" population, ITT) was defined as all randomized patients who received chemotherapy and study medication, according to randomized treatment (regardless of actual treatment received). A "per-protocol" (PP) population was defined as all patients who completed the study on day 1 and who were compliant with the study protocol (absence of major protocol violations).

RESULTS
Participant flow and recruitment
The participants' flow is shown in figure 2. A total of 570 patients were randomised, with 328 (58%) patients coming from 23 institutions from Russia, and the remaining from 35 institutions from Germany, Italy, the United Kingdom, and the Netherlands. Overall, 563 (99%) patients were treated with study medication (n= 189, 189, and 185 for palonosetron 0.25 mg, 0.75 mg, and ondansetron, respectively). Three patients were randomised to palonosetron but received ondansetron instead, one patient was randomised to ondansetron but received palonosetron, and one patient was randomised to palonosetron 0.75 mg but received 0.25 mg. The total number of patients with major protocol violations was 17 (8.9%), 15 (7.9%) and 14 (7.4%), for palonosetron 0.25, 0.75 and ondansetron, respectively. The most frequent violation was intake of rescue medication before first emetic epsode on day 1 (n=11, 10 and 6 patients, respectively).

Figure 2. Participants' flow (Study PALO-99-03)



Conduct of the study
There were 6 protocol amendments, as well as changes too the statistical analysis plan. Concerning efficacy, this included changes to the eligibility criteria, definition of the primary endpoint and populations for analysis (ITT population to be analysed according to randomized treatment, regardless of actual treatment received; PP population to exclude all patients who did not receive randomized treatment), handling of missing data, chemotherapy administration and emetogenicity, randomization method, use of concomitant medications, secondary efficacy endpoints and analysis methods. After

unblinding changes included some changes to the statistical analyses of secondary endpoints (PALO-99-03, 05).

Baseline data
Demographic and baseline disease characteristics are shown in tables 9 - 10. The majority of patients (>98%) were Caucasians.

Numbers analysed
The ITT cohort comprised 563 patients (palonosetron 0.25 mg and 0.75 mg, 189 subjects each; ondansetron 32 mg, 185 subjects). The PP cohort comprised 517 patients (N=172, 174, and 171 for palonosetron 0.25 mg, 0.75 mg, and ondansetron 32 mg, respectively).

Table 9. Demographic and baseline patient characteristics for the ITT population (study PALO-99-03)

		Palonosetron 0.25 mg N=189	**Palonosetron 0.75 mg N=189**	**Ondansetron 32 mg N=185**
Gender No. (%)	Male	54 (28.6)	51 (27.0)	52 (28.1)
	Female	135 (71.4)	138 (73.0)	133 (71.9)
Age	Mean	56.1	54.8	55.3
	Median	57.0	55.0	56.0
	Range	27, 82	29, 77	26-81
CT history No. (%)	Naïve	76 (40.2)	80 (42.3)	78 (42.2)
	Non-naïve	113 (59.8)	109 (57.7)	107 (57.8)
Renal impairment No. (%)	Yes	14 (7.4)	19 (10.1)	17 (9.2)
	No	175 (92.6)	170 (89.9)	168 (90.8)
Hepatic impairment No. (%)	Yes	27 (14.3)	27 (14.3)	24 (13.0)
	No	162 (85.7)	162 (85.7)	161 (87.0)
Cardiac impairment No. (%)	Yes	50 (26.5)	47 (24.9)	55 (29.7)
	No	139 (73.5)	142 (75.1)	130 (70.3)
Karnofsky PS	Mean	88.9	89.5	88.5
	Median	90.0	90.0	90.0
	Range	50-100	50-100	50-100
Primary cancer (multiple primaries possible)	Breast	114 (60.3)	103 (54.5)	105 (56.8)
	Lung	14 (7.4)	11 (5.8)	18 (9.7)
	Colon/rectum	18 (9.5)	13 (6.9)	11 (5.9)
	SCLC	7 (3.7)	9 (4.8)	3 (1.6)
	Gastric	6 (3.2)	6 (3.2)	6 (3.2)
	Prostate	6 (3.2)	1 (.5)	3 (1.6)
	Other	10 (5.3)	20 (10.6)	22 (11.9)

Abbreviations: [a]: includes all type of lung cancer (such as bronchial carcinoma or pulmonary carcinoma) excluding SCLC and NSCLC; CT: chemotherapy; PS: performance status; NSCLC: non-small cell lung cancer; SCLC: small cell lung cancer; Other: includes bladder cancer, Hodgkin's disease, ovarian cancer and bile duct cancer.

Table 10: Concomitant CT treatment administered on study day 1 for the ITT population (study PALO-99-03)

Substance	Palonosetron 0.25 mg N=189 No. (%)	Median dose (mg/m^2)	Palonosteron 0.75 mg N=189 No. (%)	Median dose (mg/m^2)	Ondansetron 32 mg N=185 No. (%)	Median dose (mg/m^2)
Cyclophosphamide	119 (63.0)	500	120 (63.5)	555	117 (63.2)	560
Doxorubicin	97 (51.3)	50	87 (46.0)	50	87 (47.0)	50
Cisplatin	36 (19.0)	37	33 (17.5)	36	31 (16.8)	29
Methotrexate	23 (12.2)	40	32 (16.9)	30	36 (19.5)	40
Carboplatin	15 (7.9)	290	25 (13.2)	300	25 (13.5)	300
Epirubuicin	13 (6.9)	70	17 (9.0)	60	14 (7.6)	80
Irinotecan	10 (5.3)	180	8 (4.2)	113	8 (4.3)	153
Ifosfamide	2 (1.1)	4000	0 (0.0)	-	2 (1.1)	2852
Mitoxantrone	1 (0.5)	10	1 (0.5)	12	3 (1.6)	8

Outcomes and estimation

The results of the primary analysis are shown in table 11 for the ITT and the PP populations. Non-inferiority of both palonosetron doses compared to ondansetron was shown. In secondary analyses, non-inferiority of both doses of palonosetron to ondansetron for CR was demonstrated for all daily and cumulative time periods during the study. Palonosetron 0.25 mg was superior to ondansetron with regard to CR on Days 1, 2, 3, and 4 and for each cumulative time period (i.e., 24 to 120, 0 to 48, 0 to 72, 0 to 96, and 0 to 120 hours). Superiority of palonosetron 0.25 mg to 0.75 mg was demonstrated for CR rates on day 2 and during the cumulative time periods 0 to 48 hours, 0 to 72 hours, 0 to 96 hours, and 0 to 120 hours.

A significant difference among the three treatment arms was observed in terms of time to treatment failure (first quartile 46.5, 21.0, and 19.5 hours, for palonosetron 0.25 mg, palonosetron 0.75 mg, and ondansetron, respectively, log-rank P=0.002, ITT population). Pairwise comparisons showed a statistically significant difference between palonosetron 0.25 mg *v.* ondansetron, and between the two palonosetron groups.

No significant differences were observed among the three treatment arms for the time to first administration of rescue medication.

Concerning the number of emetic episodes, more patients in the palonosetron 0.25 mg group had no emetic episodes on day 1, compared to the two other groups, respectively (85.2%, 77.8% and 71.4% for palonosetron 0.25 mg, palonosetron 0.75 mg, and ondansetron 32 mg, respectively, in the ITT population). A similar effect was observed on days 2 and 3 (87.8%, 75.7%, 69.7%, and 89.9%, 84.1%, 74.6%, for the three treatment arms on day 2 and 3, respectively). No important differences were observed between treatment arms on days 4 and 5. Overall (days 1 to 5), palonosetron 0.25mg had a significantly lower number of emetic episodes than ondansetron.

Time to first emetic episode was significantly different between treatment arms (log-rank $P< 0.001$). The first quartile time to first emetic episode was 115.1 hours, 25.2 hours and 20.5 hours for palonosetron 0.25 mg, 0.75 mg, and ondansetron 32 mg, respectively (median time to first emetic episode was longer than 120 hours for all treatment arms).

Concerning severity of nausea, significant differences among the three arms were observed on all days, except day 1. Overall, the proportion of patients without nausea was highest in the palonosetron 0.25 group and lowest in the ondasetron group.

A slightly higher patient global satisfaction was observed in the palonosetron 0.25 group compared to the other treatment groups on study days 2 (first quartile 78, 66, 52, and 83, 77, 60, for palonosetron 0.25 mg, 0.75 mg, and ondansetron on day 2 and 3, respectively). No statistically significant differences in patient global satisfaction with anti-emetic therapy were observed except on day 3.

Median quality of life scores were similar across treatment arms for nausea and vomiting during the whole study period. A group difference was observed for the total score of the time period between 24

and 96 hours (P=0.047, Kruskal-Wallis test). Further pairwise comparisons showed a significant difference for palonosetron 0.25 mg, compared to ondansetron 32 mg.

Further exploratory subgroup analyses were performed to study the consistency of the treatment effect ain different subgroups defined by gender, and chemotherapy history. Differences between palonosetron and ondansetron in favour of both palonosetron doses were observed for most efficacy parameters in both male and female patients. During the first 24 hours after chemotherapy, male gender was associated with 13.7%, 22.8%, and 14.1% higher CR rates compared to female patients for palonosetron 0.25 mg, 0.75 mg, and ondansetron, respectively. Similarly, higher CC rates, less nausea, longer time to treatment failure, longer time to first emetic episode, less rescue medication, higher patient global satisfaction, and higher quality of life scores for nausea were observed for male patients. The difference between both palonosetron groups was more pronounced in female than in male patients. Non-inferiority of both palonosetron doses compared to ondansetron in terms of CR was observed in chemotherapy naïve and non-naïve patients, except for palonosetron 0.75 mg in chemotherapy naïve patients (97.5 CI: -22.9%, 11.7%). Chemotherapy naïve patients tended to have less nausea, a higher patients global satisfaction, and higher quality of life scores than non-naïve subjects.

An exploratory logistic regression analysis was conducted to study the treatment effect after adjusting for gender, chemotherapy history and geographic region. A statistically significant effect (5% level) of palonosetron 0.25 mg was observed in the ITT population during all time periods except on day 5.

Table 11: CR during the first 24 hours after chemotherapy: PALO-99-03 (ITT cohort, N = 563)

ITT	Palonosetron 0.25 mg (N = 189)	Palonosetron 0.75 mg (N = 189)	Ondansetron 32 mg (N = 185)
CR at 24 hours No. (%)	153 (81.0)	139 (73.5)	127 (68.6)
95% CI	[74.5%, 86.1%]	[66.6%, 79.6%]	[61.4%, 75.1%]
Difference in CR (palo-ondansetron) 97.5% CI	[1.8%, 22.8%]	[-6.1%, 15.9%]	
PP	Palonosetron 0.25 mg (N = 172)	Palonosetron 0.75 mg (N = 174)	Ondansetron 32 mg (N = 171)
CR at 24 hours No. (%)	151 (87.8)	138 (79.3)	121 (70.8)
95% CI	[81.7%, 92.1%]	[72.4%, 84.9%]	[63.2%, 77.3%]
Difference in CR (palo-ondansetron) 97.5% CI	[6.9%, 27.2%]	[-2.4%, 19.5%]	

PALO-99-04

PALO-99-04 was a three-arm double-blind multicentre randomised active-controlled clinical trial of single i.v. doses of palonosetron 0.25 mg or 0.75 mg, *vs.* dolasetron 100 mg i.v., for the prevention of moderately emetogenic CINV.

METHODS

Study participants (See study PALO-99-03).

One additional exclusion criteria for study PALO-99-04 was QTc > 500 msec at baseline.

Treatments
Patients randomised to palonosetron 0.25 mg, or 0.75 mg were adminstered palonostern intravenously as a 30 second bolus, followed by a 30 minutes infusion of 0.9% saline. Rescue medication for the treatment of nausea and vomiting after chemotherapy, with the exception of study drugs, was permitted at the investigator's discretion. The study drug was adminstered 30 minutes prior to chemotherapy.

Objectives
The primary objective was to compare the efficacy of single-doses of palonosetron 0.25 or 0.75 mg, to dolasetron 100 mg i.v. in preventing moderately emetogenic CINV. The secondary objectives were to evaluate the safety and tolerability of palonosetron, and the effect of anti-emetic control on quality of life.

Outcomes / endpoints
The primary efficacy endpoint was complete response (CR) during the first 24 hours after administration of chemotherapy. CR was defined as no emetic episodes and no rescue medication. An emetic episode was defined as one occurrence of vomiting or a sequence of occurrences in very close succession not relieved by a period of relaxation, any number of occurrences of unproductive emesis (retches) in a unique 5-minute period, or an episode of retching of less than 5 minutes duration combined with vomiting not relieved by a period of relaxation. Relaxation between episodes was defined as a period of at least 1 minute. Diaries were used to record emetic episodes, use of rescue medication and severity of nausea, at 24 hour intervals for up to 120 hours.
Secondary endpoints: see study PALO-99-04.

Sample size
A sample size of approximately 567 patients (see sample size section for study PALO-99-03). Following a protocol amendment, the sample size was increased to approximately 648 patients (216 patients per arm). This was due to the need to exclude all patients from non compliant centres as a result of quality assurance audits.

Randomisation
Randomization (as amended) was stratified by gender, previous chemotherapy history (naive *v.* non-naive), using an adaptive dynamic method (minimisation). Use of i.v. dexamethasone was added through an amendment as a stratification factor at randomization.

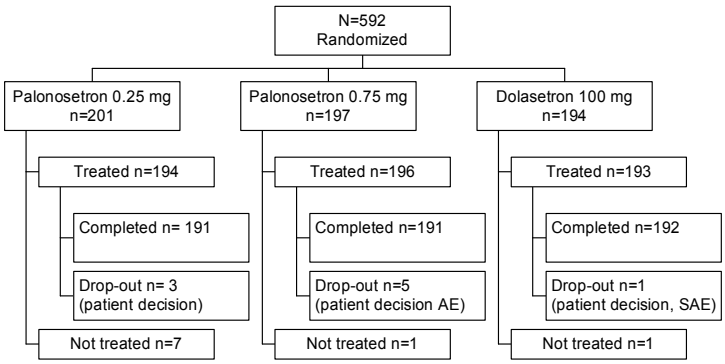
Blinding (masking)
The trial was double-blind.

Statistical methods
See study PALO-99-03.

RESULTS

Participant flow and recruitment
The participants' flow is shown in figure 3. A total of 592 patients were randomised, with 331 (56%) patients coming from 20 institutions from Mexico, (22%) patients coming from 28 institutions from the US and (22%) patients coming from 13 institutions from California. Overall, 583 (98%) patients were treated with study medication (n= 194, 196, and 193 for palonosetron 0.25 mg, 0.75 mg, and dolasetron, respectively). One patients were randomised to palonosetron 0.25 mg but received dolasetron instead. The total number of patients with major protocol violations was 33 (17.5%), 38 (20.1%) and 35 (18.3%), for palonosetron 0.25, 0.75 and dolasetron, respectively. The most frequent violation was intake of rescue medication before first emetic episode on day 1 (n=21, 23 and 16 patients, respectively).

Figure 3: Participants' flow (Study PALO-99-04)



Conduct of the study

There were 7 protocol amendments, as well as changes too the statistical analysis plan. Concerning efficacy, this included changes to the sample size calculation, statistical analysis, eligibility criteria, selection criteria, definition of the primary endpoint and populations for analysis (ITT population to be analysed according to randomized treatment, regardless of actual treatment received; PP population to exclude all patients who did not receive randomized treatment), handling of missing data, chemotherapy administration and emetogenicity, randomization method, use of concomitant medications, secondary efficacy endpoints, analysis methods, patients monitoring and centers. After unblinding changes included some changes to the statistical analyses of primary and secondary endpoints.

Baseline data

Demographic and baseline disease characteristics are shown in tables 12-14. More than 60% of patients were Hispanic and more than 30% were Caucasian.

Numbers analysed

All patients from one center (13 patients) were excluded from the ITT cohort due to inconsistencies and doubtful data but were analysed in the safety cohort. Therefore, the ITT cohort comprised 569 patients (N=189, 189, and 191 for palonosetron 0.25 mg, 0.75 mg, and dolasetron 32 mg, respectively). The PP cohort comprised 463 patients (N=156, 151, and 156 for palonosetron 0.25 mg, 0.75 mg, and dolasetron 32 mg, respectively). The number of patients with corticosteroid use was low since the amendment allowing the use of dexamethasone became effective late in the study, 5 months before study end.

Table 12: Demographic and baseline patient characteristics for the ITT population (study PALO-99-04)

		Palonosetron 0.25 mg N=189	Palonosetron 0.75 mg N=189	Dolasetron 100 mg N=191
Gender No. (%)	Male	34 (18.0)	33 (17.5)	35 (18.3)
	Female	155 (82.0)	156 (82.5)	156 (81.7)
Age	Mean	53.3	55.2	53.6
	Median	52.0	56.0	52.0
	Range	21, 86	18, 84	18, 97
CT history No. (%)	Naïve	124 (65.6)	131 (69.3)	125 (65.4)
	Non-naïve	65 (34.4)	58 (30.7)	66 (34.6)
Renal impairment No. (%)	Yes	14 (7.4)	19 (10.1)	17 (9.2)
	No	175 (92.6)	170 (89.9)	168 (90.8)
Hepatic impairment No. (%)	Yes	27 (14.3)	27 (14.3)	24 (13.0)
	No	162 (85.7)	162 (85.7)	161 (87.0)
Cardiac impairment No. (%)	Yes	50 (26.5)	47 (24.9)	55 (29.7)
	No	139 (73.5)	142 (75.1)	130 (70.3)
Karnofsky PS	Mean	94.7	93.4	94.2
	Median	100.0	100.0	100.0
	Range	70-100	50-100	60-100
Corticosteroid use (%)	Yes	11 (5.8)	12 (6.3)	8 (4.2)
	No	178 (94.2)	177 (93.7)	183 (95.8)

Abbreviations: CT: chemotherapy; PS: performance status

Table 13: Most frequent primary cancers (multiple primaries possible) - Study PALO-99-04 (safety cohort, N = 582)

	Palonosetron 0.25 mg N=193	Palonosetron 0.75 mg N=195	Dolasetron 100 mg N=194
Breast	131 (67.9)	116 (59.5)	131 (67.5)
Non Hodgkin's lymphoma	8 (4.1)	13 (6.7)	8 (4.1)
Lung	8 (4.1)	10 (5.1)	7 (3.6)
NSCLC	5 (2.6)	2 (1.0)	2 (1.0)
SCLC	3 (1.6)	6 (3.1)	4 (2.1)
Ovarian	4 (2.1)	9 (4.6)	3 (1.5)
Cervical cancer carcinoma	3 (1.6)	2 (1.0)	2 (1.0)
Other	10 (5.3)	20 (10.6)	22 (11.9)

Abbreviations: [a]: includes all type of lung cancer (such as bronchial carcinoma or pulmonary carcinoma) excluding SCLC and NSCLC; NSCLC: non-small cell lung cancer; SCLC: small cell lung cancer; Other: includes colon cancer, prostate cancer, acute lymphocytic leukemia.

Table 14. Concomitant CT treatment administered on study day 1 for the ITT population (Study PALO-99-04)

Substance	Palonosetron 0.25 mg N=189 No. (%)	Median dose (mg/m^2)	Palonosetron 0.75 mg N=189 No. (%)	Median dose (mg/m^2)	Dolasetron 100 mg N=191 No. (%)	Median dose (mg/m^2)
Cyclophosphamide	138 (73.0)	500	129 (68.3)	600	146 (76.4)	500
Doxorubicin	91 (48.1)	50	77 (40.7)	50	93 (48.7)	50
Epirubicin	39 (20.6)	75	44 (23.3)	75	43 (22.5)	75
Carboplatin	30 (15.9)	87	38 (20.1)	255	26 (13.6)	300
Cisplatin	14 (7.4)	40	8 (4.2)	40	7 (3.7)	50
Methotrexate	7 (3.7)	40	9 (4.8)	40	6 (3.1)	40
Mitoxantrone	4 (2.1)	9	5 (2.6)	10	8 (4.2)	12
Irinotecan	2 (1.1)	113	4 (2.1)	113	3 (1.6)	100
Ifosfamide	2 (1.1)	2250	0 (0.0)	-	1 (0.5)	2850
Idarubicin	2 (1.1)	15	0 (0.0)	-	0 (0.0)	-

Outcomes and estimation

The results of the primary analysis are shown in table 15 for the ITT and the PP populations. Non-inferiority of both palonosetron doses compared to dolasetron was shown. In secondary analyses, non-inferiority of both doses of palonosetron to dolasetron for CR was demonstrated for all daily and cumulative time periods during the study. Palonosetron 0.25 mg was superior to dolasetron with regard to CR on Days 2 and 3 and for the cumulative time period 0 to 48, 0 to 120 and 24 to 120 hours. Palonosetron 0.75 mg was superior to dolasetron with regard to CR on Days 2, 3 and 4 and for the cumulative time period 0 to 72, 0 to 96, 0 to 120 and 24 to 120 hours periods. Superiority of palonosetron 0.25 mg to 0.75 mg was demonstrated for CR rates on day 1.

A significant difference among the three treatment arms was observed (log-rank P=0.025, ITT population, median times to treatment failure were 51.1, 52.8, and 24.6 hours, for palonosetron .25 mg, palonosetron .75 mg, and dolasetron, respectively). Pairwise comparisons showed a statistically significant difference between both palonosetron doses and dolasetron. No significant differences were observed between the palonosetron groups.

No significant differences were observed among the three treatment arms for the time to first administration of rescue medication.

Concerning the number of emetic episodes, more patients in the palonosetron 0.25 mg group had no emetic episodes on day 1, compared to the two other groups, respectively (72.0%, 65.18% and 58.6% for palonosetron 0.25 mg, palonosetron 0.75 mg, and dolasetron 100 mg, respectively, in the ITT population). On day 2, 4 and 5, the rates of patients without emetic episodes were comparable between the palonosetron groups (70.9%, 75.1%; 89.9%, 88.9% and 95.8% and 92.6%, for palonosetron 0.25 mg, palonosetron 0.75 mg arms on day 2, 4 and 5, respectively). On day 3, the proportion of patients without emetic episodes was higher in the palonosetron 0.75 mg group compared to palonosetron 0.25 mg (84.1% vs 77.8%, respectively). Overall (days 1 to 5), both palonosetron doses had a significantly lower number of emetic episodes than dolasetron.

Time to first emetic episode was significantly different between treatment arms (log-rank $P = 0.008$). The first quartile time to first emetic episode was 13.5 hours, 9.8 hours and 7.9 hours for palonosetron 0.25 mg, 0.75 mg, and dplasetron 100 mg, respectively (median time to first emetic episode was longer than 120 hours for both palonosetron arms, and 41.5 hours for dolasetron arm).

Concerning severity of nausea, significant differences among the three arms were observed on study day 2, 3 and 4. Overall, the proportion of patients without nausea was highest in the palonosetron groups compared with the dolasetron group.

A slightly higher patient global satisfaction was observed in the palonosetron groups compared to dolasetron 100 mg. Statistically significant differences in patient global satisfaction with anti-emetic therapy were observed on day 2 and 4.

Median quality of life scores for nausea during the period 0-24 hours and 24-96 hours were higher in both palonosetron groups compared to dolasetron 100 mg. No differences on the quality of life scores for vomiting were seen. Statistical significant differences were seen between the treatment groups for quality of life scores during 24-96 hours after chemotherapy for nausea (P = 0.013) and, as a result of nausea, for the total score (P=0.016, Kruskal-Wallis test). Further pairwise comparisons showed a significant difference between both palonosetron doses compared to dolasetron for the quality of life scores during the 24-96 hours period for nausea, and for the total score.

Further exploratory subgroup analyses were performed to study the consistency of the treatment effect in different subgroups defined by gender, and chemotherapy history. A consistent treatment group difference was found in male patients with the palonosetron 0.25mg dose showing better efficacy than the 0.75 mg palonosetron and the 100 mg dolasetron doses. During the first 24 hours after chemotherapy, male gender was associated with 30.8%, 7.8%, and 12.3% higher CR rates compared to female patients for palonosetron 0.25 mg, 0.75 mg, and dolasetron 100 mg, respectively. Similarly, higher CC rates, less nausea, longer time to treatment failure, longer time to first emetic episode, longer time to first administration of rescue medication, less rescue medication, higher patient global satisfaction, and higher quality of life scores for nausea were observed for male patients. Non-inferiority of both palonosetron doses compared to dolasetron in terms of CR was observed in chemotherapy naïve patients only. Chemotherapy non-naïve patients tended to have longer time to treatment failure, less rescue medication, and higher quality of life scores than naïve patients.

The relative frequency of patients with CR during the first 24 hours after chemotherapy was higher in patients with corticoid use compared to patients without corticoid use the palonosetron 0.25 mg and dolasetron groups. In the palonosetron 0.75 mg group, the percentage of patients with CR was higher in patients without corticosteroids.
An exploratory logistic regression analysis was conducted to study the treatment effect after adjusting for gender, chemotherapy history and geographic region. A statistically significant effect (5% level) of palonosetron 0.25 mg was observed in the ITT population on days 2 and 3 as well as during all cumulative time periods.

Table 15: CR during the first 24 hours after chemotherapy: PALO-99-04 (ITT cohort, N = 569)

ITT	**Palonosteron 0.25 mg** (N = 189)	**Palonosetron 0.75 mg** (N = 189)	**Dolasetron 100 mg** (N = 191)
CR at 24 hours No. (%)	119 (63.0)	108 (57.1)	101 (52.9)
95% CI	[55.6%, 69.8%]	[49.8%, 64.26%]	[45.6%, 60.1%]
Difference in CR (palonosetron-dolasetron) 97.5% CI	[-1.7%, 21.9%]	[-7.7%, 16.2%]	
PP	Palonosetron 0.25 mg (N = 156)	Palonosetron 0.75 mg (N = 151)	Dolasetron 100 mg (N = 156)
CR at 24 hours No. (%)	112 (71.8)	100 (66.2)	93 (59.6)
95% CI	[63.9%, 78.6%]	[58.0%, 73.6%]	[51.5%, 67.3%]
Difference in CR (palonosetron-dolasetron) 97.5% CI	[-0.4%, 24.8%]	[-6.4%, 19.6%]	

PALO-99-05

PALO-99-05 was a three-arm double-blind multicentre randomised active-controlled clinical trial of single i.v doses of palonosetron 0.25 mg or 0.75 mg *vs.* ondansetron 32 mg i.v, for the prevention of *highly* emetogenic CINV.

METHODS

Study Participants
The main inclusion criteria were similar to PALO-99-03, except that patients had to be scheduled to receive a single dose of at least one of the following agents on day 1: cisplatin ≥ 60 mg/m^2 (adminstered over 1-4 hours for doses < 70 mg/m^2, administered over 2-4 hours for doses ≥ 70 mg/m^2), cyclophosphamide ≥ 1500 mg/m^2, carmustine (BCNU) > 250 mg/m^2, dacarbazine (DTIC), mechlorethamine (nitrogen mustard). The administration of the main emetogenic agent following the classification proposed by Hesketh *et al*. [32] on study day 1 should not extend beyond 4 hours. The main criteria for exclusion were similar to PALO-99-03. Patients who were scheduled to receive at any time during days 2-6 of the study DTIC, mechloretamine, cisplatin, cyclophosphamide > 1500 mg/m^2, BCNU > 250 mg/m^2, lomustine (CCNU) > 60 mg/m^2, or any other chemotherapeutic agent with an emetogenicity level 4 or above were not eligible.

Treatments
Randomized patients were administered i.v. palonosetron 0.25 mg, 0.75 mg as a 30 second bolus, or ondansetron 32 mg over 15 minutes, in a blinded fashion 30 minutes prior to chemotherapy (see PALO-99-03).

Objectives
The primary and secondary objectives were similar to those of PALO-99-03, but for prevention of highly emetogenic CINV.

Outcomes / endpoints
The primary efficacy endpoint was complete response (CR) during the first 24 hours after administration of chemotherapy. For response definition and secondary endpoints, see PALO-99-03.

Sample size
Initially, a sample size of approximately 669 patients (223 patients per arm) was calculated based on an assumed response rate of 50%, a difference of no more than 15% in CR proportion, and setting α=0.0125 (*i.e.*, α=0.025/2 to adjust for the two treatment comparisons) for a one-sided test of equivalence, 80% power for each comparison, and a 5% dropout. Prior to unblinding, an additional 11 patients were randomized to allow for sites excluded from the study.

Randomization
See study PALO-99-04.

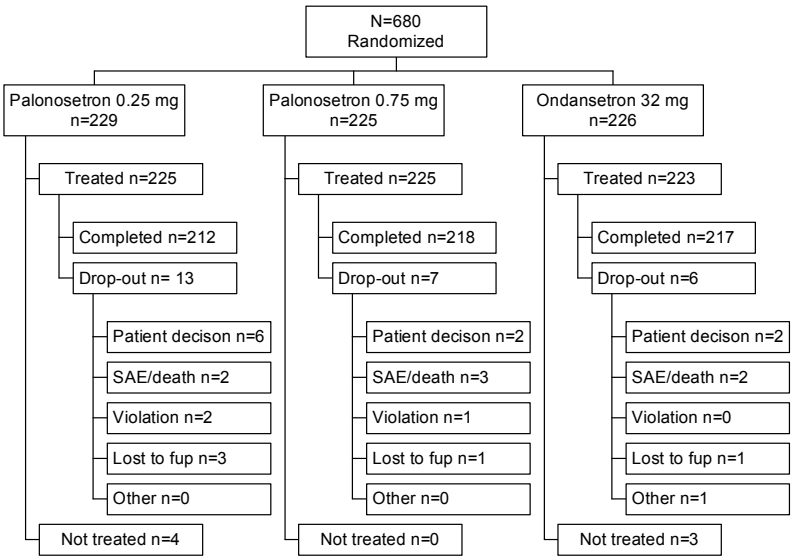
Blinding (masking)
The trial was double-blind.

Statistical methods
See study PALO-99-03.

RESULTS

Participant flow and recruitment
The participants' flow is shown in figure 4. Seventy-six (76) centres enrolled a total of 680 patients in the study, with 270 (40%) of patients from 18 institutions from Russia, 242 (36%) patients from 18 institutions from Mexico, and the remainder 168 (25%) from 40 institutions from Western Europe, the U.S.A, and Canada. Overall, 7 patients did not receive study medication.

Figure 4. Participants' flow (Study PALO-99-05)



Conduct of the study

There were several protocol amendments and changes to the statistical analyses of secondary endpoints similar to those described fro PALO-99-03. Prior to unblinding, all patients from one site were excluded (N=6).

Baseline data

Demographic and baseline disease characteristics are shown in tables 16 - 18. The majority of patients (60%) were Caucasians or Hispanic (36%).

Numbers analysed

The ITT cohort comprised 667 patients (palonosetron 0.25 mg and 0.75 mg, 223 subjects each; ondansetron 32 mg, 221 subjects). The PP cohort comprised 572 patients (N=185, 191, and 196 for palonosetron 0.25 mg, 0.75 mg, and ondansetron 32 mg, respectively).

Table 16: Demographic and baseline patient characteristics for the ITT population (study PALO-99-05)

		Palonosetron 0.25 mg N=223	Palonosetron 0.75 mg N=223	Ondansetron 32 mg N=221
Gender No. (%)	Male	108 (48.8)	110 (49.3)	108 (48.9)
	Female	115 (51.6)	113 (50.7)	113 (51.1)
Age	Mean	53.4	50.6	50.9
	Median	55.0	52.0	52.0
	Range	18-86	18-82	18-86
CT naïve	No. (%)	133 (59.6)	129 (57.8)	131 (59.3)
Corticosteroid use	No. (%)	150 (67.3)	150 (67.3)	147 (66.5)
Renal impairment	No. (%)	12 (5.4)	17 (7.6)	17 (7.7)
Hepatic impairment	No. (%)	15 (6.7)	21 (9.4)	14 (6.3)
Cardiac impairment	No. (%)	38 (17.0)	43 (19.3)	36 (16.3)
Karnofsky PS	Mean	88.4	88.7	90.1
	Median	90.0	90.0	90.0
	Range	50-100	40-100	60-100

Table 17: Most frequent primary cancers (multiple primaries possible) for the randomized and treated population (N=673, study PALO-99-05)

	Palonosetron 0.25 mg N=225	**Palonosetron 0.75 mg N=225**	**Ondansetron 32 mg N=223**
Ovarian	36 (16.0)	40 (17.8)	39 (17.5)
HD	22 (9.8)	14 (6.2)	17 (7.6)
Lung [a]	20 (8.9)	18 (8.0)	20 (9.0)
Breast	12 (5.3)	5 (2.2)	14 (6.3)
Gastric	8 (3.6)	11 (4.9)	14 (6.3)

Abbreviations: [a]: includes all type of lung cancer (such as bronchial carcinoma or pulmonary carcinoma) excluding SCLC and NSCLC; HD: Hodgkin's disease.
Note: frequencies not reported for less frequent primaries (head & neck, nasopharinx, oesophagus, SCLC, NSCLC, pancreas, endometrium, cervix, testis, bladder, Non-Hodgkin's lymphoma, melanoma, and myeloma).

Table 18: Concomitant CT treatment administered on study day 1 (multiple answers possible) for the ITT population (Study PALO-99-05)

Substance	Palonosetron 0.25 mg N=223 No. (%)	Median dose (mg/m^2)	Palonosteron 0.75 mg N=223 No. (%)	Median dose (mg/m^2)	Ondansetron 32 mg N=221 No. (%)	Median dose (mg/m^2)
Cisplatin	184 (82.5)	75	189 (84.8)	80	181 (81.9)	80
Cyclophosphamide	57 (25.6)	700	53 (23.8)	600	59 (26.7)	700
Dacarbazine	28 (12.6)	375	24 (10.8)	388	30 (13.6)	467
Carmustine	1 (0.4)	120	-	-	-	-

Outcomes and estimation

The results of the primary analysis are shown in table 19 for the ITT and the PP populations. Non-inferiority of both palonosetron doses compared to ondansetron was shown. In secondary analyses, non-inferiority of both doses of palonosetron to ondansetron for CR was demonstrated for all daily and cumulative time periods during the study. The two palonosetron doses were similar (with differences in PR proportions within ± 15%), except for the 0 to 24 hours period where a larger than 15% difference favouring the 0.75 dose could not be excluded (95% CI for difference in proportion of CR for palonosetron 0.75 mg minus 0.25 mg: -3.5%, 15.7%).

No statistically significant differences in the complete control proportions were observed across the three groups during any of the cumulative study periods. Significant differences (favouring palonosetron .75 mg) were only observed on day 4.

No statistically significant differences were observed among the three treatment arms in terms of time to treatment failure (median 45.3, 45.6, and 34.2 hours, for palonosetron 0.25 mg, palonosetron 0.75 mg, and ondansetron, respectively, log-rank P=0.096, ITT population), and time to first administration of rescue medication.

Concerning the number of emetic episodes, statistically significant differences in the number of emetic episodes were observed on day1 and for the overall time period 0 to 120. More patients in the palonosetron 0.75 mg group had no emetic episodes on day 1, compared to the two other groups, respectively (68.2%, 72.2% and 60.2% for palonosetron 0.25 mg, palonosetron 0.75 mg, and ondansetron 32 mg, respectively, in the ITT population). No significant differences were observed on study days 2 to 5.

Time to first emetic episode was significantly different between treatment arms (log-rank $P< 0.012$). The first quartile time to first emetic episode was 19.5 hours, 21.0 hours and 17.3 hours for palonosetron 0.25 mg, 0.75 mg, and ondansetron 32 mg, respectively (the median time to first emetic episode was longer than 120 hours for all palonosetron arms, and 42.7 hours for the ondansetron arm).

No significant differences were observed for severity of nausea among the three arms on any of the study days.

No statistically significant differences in patient global satisfaction with anti-emetic therapy were observed except on any study days.

No differences in quality of life scores were observed across treatment arms for nausea and vomiting during the whole study period.

Further exploratory subgroup analyses were performed to study the consistency of the treatment effect in different subgroups defined by gender, chemotherapy history, and corticosteroid use. In female patents palonosetron 0.75 had significantly higher CR proportion compared to ondansetron 32 mg. No significant differences were observed for male patients among the three treatment arms. In chemotherapy naïve patients the palonosetron 0.75 mg group showed a significantly higher response rate than the other arms, whereas the proportion of CR was similar in non-naïve patients, during the first 24 hours. Concerning further time points, the estimated lower limits of the 97.5% confidence intervals were within the pre-defined non-inferiority margin for both palonosetron doses for all cumulative and daily intervals (except for ondansetron 0.25 mg in naïve patients on days 4 and 5). Concerning corticosteroid use, non-inferiority to ondansetron 32 mg was shown for both doses, except for patients randomized to palonosetron 0.25 mg without corticosteroid use. In patients without corticosteroid use, palonosetron 0.75 mg was significantly better than 0.25 mg. For further study days, non-inferiority to ondansetron 32 mg was observed for both palonosetron doses in patients with corticosteroid use, but not for palonosetron 0.25 mg in without corticosteroid use on days 2 to 5 or for palonosetron 0.75 mg for days 2 to 4.

Table 19: CR during the first 24 hours after chemotherapy: PALO-99-05 (ITT cohort, N = 667)

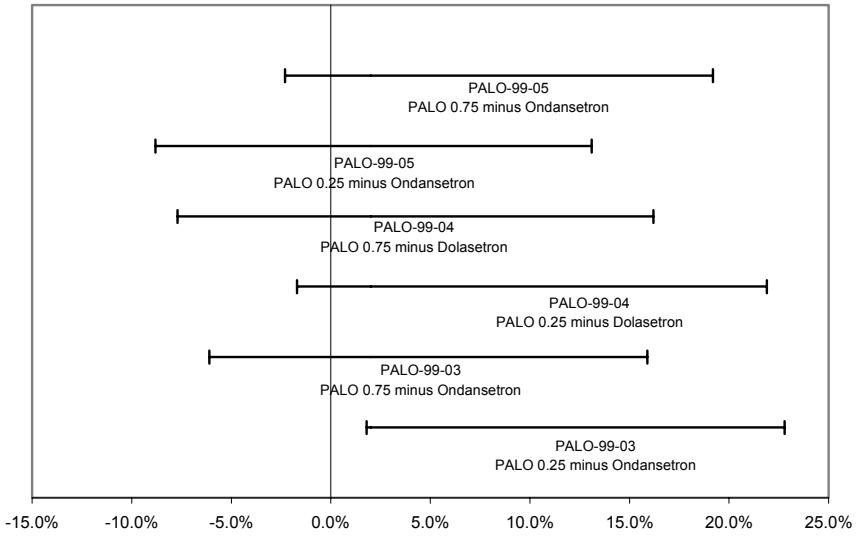
ITT	Palonosetron 0.25 mg (N = 223)	Palonosetron 0.75 mg (N = 223)	Ondansetron 32 mg (N = 221)
CR at 24 hours No. (%) 95% CI	132 (59.2) [52.4%, 65.6%]	146 (65.5) [58.8%, 71.6%]	126 (57.0) [50.2%, 63.6%]
Difference in CR (palonosetron-ondansetron) 97.5% CI	[-8.8%, 13.1%]	[-2.3%, 19.2%]	
PP	**Palonosetron 0.25 mg (N = 185)**	**Palonosetron 0.75 mg (N = 191)**	**Ondansetron 32 mg (N = 196)**
CR at 24 hours No. (%) 95% CI	128 (69.2) [61.9%, 75.6%]	139 (72.8) [65.8%, 78.8%]	124 (63.3) [56.1%, 69.9%]
Difference in CR (palonosetron-ondansetron) 97.5% CI	[-5.4%, 17.3%]	[-1.6%, 20.6%]	

- Analysis performed across trials

A comparison of efficacy results of the pivotal studies (PALO-99-03, PALO-99-04, PALO-99-05) was performed.

Complete Response rates during the first 24 hours
The 97.5% confidence intervals CIs for the difference in CR rates during the first 24 hours after chemotherapy between the palonosetron doses and active comparators are shown in Figure 5 for studies PALO-99-03, PALO-99-04, and PALO-99-05 (ITT Cohorts: N = 563, N = 569, and N = 667):

Figure 5: Difference in CR rates during the first 24 hours in pivotal studies (ITT cohorts)



In the moderately emetogenic CINV studies, the percentage of patients with CR during the first 24 hours after chemotherapy was greatest in the palonosetron 0.25 mg group (range 63.0 % to 81.0 %) and least in the active comparator group (dolasetron 52.9%, ondansetron 68.6 %). In contrast, in the prevention of highly emetogenic CINV the greatest percentage of patients with CR during the first 24 hours after chemotherapy was seen in the palonosetron 0.75 mg group (65.5%). The CR rates for palonosetron 0.25 mg and ondansetron were comparable (59, 2% vs. 57.0%, respectively).
In all three studies, both palonosetron doses were non-inferior to ondansetron and dolasetron for the prevention of moderately as well as highly emetogenic CINV in terms of CR rates during the first 24 hours after chemotherapy. In study PALO-99-03 palonosetron 0.25 mg was superior to ondansetron.

Complete Response over time (by day)
CR rates by day for the 0 to 24, 24 to 48, 48 to 72, 72 to 96, and 96 to 120 hour time periods (study days 1, 2, 3, 4, and 5, respectively) were measured for studies PALO-99-03, PALO-99-04 and PALO-99-05. In all three studies CR rates by day were greater for palonosetron, regardless of the dose, than for the respective active comparator for study days 1 through 5. In all three studies the lower limit of the 97.5 % CI for the CR rates of both palonosetron doses was above -15% on each study day, indicating non-inferiority of both doses of palonosetron to the respective comparators with respect to CR for all daily periods. In Study PALO-99-03, statistically superior CR rates were observed for palonosetron 0.25 mg compared to ondansetron on study days 1, 2, 3, and 4, since the 97.5% CIs for the difference between the two treatments did not include zero. In Study PALO-99-04, statistical superiority to dolasetron was shown for palonosetron 0.25 mg on study days 2 and 3 and for palonosetron 0.75 mg on study days 2, 3, and 4. In the highly emetogenic CINV study, the 97.5% CI of the comparison between palonosetron 0.25 mg and ondansetron shifted toward positive values on study days 2 and 3, showing a trend towards greater efficacy of palonosetron on these days. Overall, no substantial differences were shown in daily CR rates between the two palonosetron doses in all three studies (data not shown).
Complete Response over time (cumulative time periods)
In all three studies, the relative frequency of patients with CR during each cumulative time period over the five-day observation period was greater in both palonosetron groups compared to active comparators. In all three studies, non-inferiority of both palonosetron doses to ondansetron and dolasetron, respectively, was shown during all cumulative time periods after chemotherapy since the lower limit of the 97.5% CI was above the pre-set threshold of -15%. Furthermore, in the moderately emetogenic CINV study PALO-99-03, the 97.5% CIs for the comparison of palonosetron 0.25 mg with ondansetron did not include zero, indicating statistically superior CR rates with palonosetron 0.25 mg compared to ondansetron for all cumulative time periods. In study PALO-99-04, statistical superiority of palonosetron 0.25 mg to dolasetron for CR was observed for the 0 to 48, 0 to 120, and

24 to 120 hour periods and for palonosetron 0.75 mg for the 0 to 72, 0 to 96, 0 to 120, and 24 to 120 hour periods. Results for palonosetron 0.25 mg are shown in table 20 - 22.

Complete Control over time (by day)
In all three studies, the Complete Control rates by day were greater on both palonosetron doses than on the active comparators throughout the observation period (data not shown).

Complete Control over time (cumulative time periods)
In all three studies, the cumulative Complete Control rates were greater on both palonosetron doses than on the active comparator throughout the observation period. The difference between palonosetron and active comparator was statistically significant in Study PALO-99-03 for palonosetron 0.25 mg and in Study PALO-99-04 for both palonosetron doses, during all cumulative periods. Apart from 0-48 and 0-72 hours in PALO-99-03, there were no statistical differences in cumulative Complete Control rates between the two doses of palonosetron. Results for palonosetron 0.25 mg are shown in table 20 - 22.

Table 20: Percentage of patients responding by treatment group and phase (ITT cohort)
Study PALO-99-03

	palonosetron 0.25 mg (N= 189)	**ondansetron 32 mg (N= 185)**	**Delta**	
	%	%	%	
Complete Response (no emesis and no rescue medication)				**97.5% CI** [a]
0 – 24 hours	81.0	68.6	12.4	[1.8%, 22.8%]
24 – 120 hours	74.1	55.1	19.0	[7.5%, 30.3%]
0 – 120 hours	69.3	50.3	19.0	[7.4%, 30.7%]
Complete Control (CR and no more than mild nausea)				**p-value** [b]
0 – 24 hours	76.2	65.4	10.8	NS
24 – 120 hours	66.7	50.3	16.4	0.001
0 – 120 hours	63.0	44.9	18.1	0.001
No nausea (Likert Scale [c]**)**				**p-value** [b]
0 – 24 hours	60.3	56.8	3.5	NS
24 – 120 hours	51.9	39.5	12.4	NS
0 – 120 hours	45.0	36.2	8.8	NS

[a] The study was designed to show non-inferiority. A lower bound greater than –15% demonstrates non-inferiority between Palonosetron and comparator.
[b] Chi-square test. Significance level at α=0.05.
[c] 4- point Likert scale measuring none, mild, moderate or severe nausea.
Abbreviation: NS: non statistically significant.

Table 21: Percentage of patients responding by treatment group and phase (ITT cohort)
Study PALO-99-04

	palonosetron 0.25 mg (N= 185)	dolasetron 100 mg (N= 191)	Delta	
	%	%	%	
Complete Response (no emesis and no rescue medication)				**97.5% CI** [a]
0 – 24 hours	63.0	52.9	10.1	[-1.7%, 21.9%]
24 – 120 hours	54.0	38.7	15.3	[3.4%, 27.1%]
0 – 120 hours	46.0	34.0	12.0	[0.3%, 23.7%]
Complete Control (CR and no more than mild nausea)				**p-value** [b]
0 – 24 hours	57.1	47.6	9.5	NS
24 – 120 hours	48.1	36.1	12.0	0.018
0 – 120 hours	41.8	30.9	10.9	0.027
No nausea (Likert Scale [c]**)**				**p-value** [b]
0 – 24 hours	48.7	41.4	7.3	NS
24 – 120 hours	41.8	26.2	15.6	0.001
0 – 120 hours	33.9	22.5	11.4	0.014

[a] The study was designed to show non-inferiority. A lower bound greater than –15% demonstrates non-inferiority between Palonosetron and comparator.
[b] Chi-square test. Significance level at α=0.05.
[c] 4- point Likert scale measuring none, mild, moderate or severe nausea.
Abbreviation: NS: non statistically significant.

Table 22: Percentage of patients responding by treatment group and phase (ITT cohort)
Study PALO-99-05

	palonosetron 0.25 mg (N= 223)	ondansetron 32 mg (N= 221)	Delta	
	%	%	%	
Complete Response (no emesis and no rescue medication)				**97.5% CI** [a]
0 – 24 hours	59.2	57.0	2.2	[-8.8%, 13.1%]
24 – 120 hours	45.3	38.9	6.4	[-4.6%, 17.3%]
0 – 120 hours	40.8	33.0	7.8	[-2.9%, 18.5%]
Complete Control (CR and no more than mild nausea)				**p-value** [b]
0 – 24 hours	56.5	51.6	4.9	NS
24 – 120 hours	40.8	35.3	5.5	NS
0 – 120 hours	37.7	29.0	8.7	NS
No nausea (Likert Scale [c]**)**				**p-value** [b]
0 – 24 hours	53.8	49.3	4.5	NS
24 – 120 hours	35.4	32.1	3.3	NS
0 – 120 hours	33.6	32.1	1.5	NS

[a] The study was designed to show non-inferiority. A lower bound greater than -15% demonstrates non-inferiority between palonosetron and comparator.
[b] Chi-square test. Significance level at α=0.05.
[c] 4- point Likert scale measuring none, mild, moderate or severe nausea.
Abbreviation: NS: non statistically significant

The results of the secondary analysis on the number of emetic episodes, severity of nausea, number of patients with rescue medication, time to treatment failure, were in accordance with the results obtained form the primary analysis (data not shown).
With regard to patient's global satisfaction and quality of life, statistically significant differences between treatments were registered only in the moderately emetogenic CINV studies, during the delayed phase of emesis (data not shown).

- Supportive study

PALO-99-06

PALO-99-06 was a multicentre, open-label, repeat-cycle study to assess the safety and efficacy of i.v. palonosetron 0.75 mg for the prevention of chemotherapy-induced nausea and vomiting in repeated chemotherapy cycles. Patients having completed one of the phase III studies (i.e., PALO-99-03, PALO-99-04, or PALO-99-05), and who were scheduled to receive at least one additional chemotherapy cycle, could have been enrolled in study PALO-99-06.
At each repeated chemotherapy cycle, a single dose of palonosetron 0.75 mg was administered 30 minutes prior to administration of the chemotherapeutic treatment (study day 1). At the discretion of the investigator, a single i.v. dose of 20 mg dexamethasone could be administered 15 minutes before the start of chemotherapy. For each cycle, a 14-day follow-up period was conducted.
The primary objective of this study was related to safety aspects. All efficacy variables were secondary objectives.
A total of 905 patients were enrolled, among which 895 were treated with study medication in at least one cycle of chemotherapy for a total of 1,733 cycles.
The ITT cohort comprised 875 subjects with 1667 cycles. Rescue medication was used in fewer than 40% of cycles during the study.
The complete response rates achieved during the first 24 hours ranged from 55.0% to 64.7% over the cycles evaluated. No safety issues were raised.

In addition to the overall cycle analysis, a separate analysis for the first five cycles was performed to analyse the persistence of treatment effects. Overall, efficacy persisted throughout subsequent cycles in terms of CR. As for the number of emetic episodes, in the acute phase (0 to 24 hours), in over 80% of cycles patients had zero or one episode of emesis (combined total: 1,334 cycles). In subsequent 24-hour intervals, the proportion of cycles in which patients had no emetic episodes steadily increased from 74.1% during the 24- to 48 hour interval to 91.1% during the 96- to 120 hour interval.
Approximately half of all patients had no nausea for the first 48 hours after administration of palonosetron, and the proportion of patients without nausea increased steadily in each successive 24-hour period.

- Discussion on clinical efficacy

The dose response studies were adequate and demonstrated that palonosetron behaves like other related drugs in having a plateau-like dose response curve [30, 31]. The dose chosen (3 µg/kg, approximately equivalent to a 0.25 mg fixed dose) was the lowest effective dose on the dose-response plateau.

In double-blind, randomised controlled trials, a single i.v. dose of palonosetron 0.25 mg was non inferior to ondansetron 32 mg (study PALO-99-03) and to dolasetron 100 mg (study PALO-99-04) in the prevention of acute (first 24 hours after chemotherapy) nausea and vomiting associated with moderately emetogenic cancer chemotherapy. Moreover, it was possible to switch from non-inferiority to superiority when palonosetron 0.25 mg was compared to ondansetron in relation to Complete Response rates.
In a double-blind, randomised controlled trial (study PALO-99-05), palonosetron 0.25 mg was non inferior to ondansetron 32 mg in the prevention of acute nausea and vomiting associated with highly emetogenic cancer chemotherapy. Additional supportive evidence of efficacy in patients receiving highly emetogenic chemotherapy was provided by a phase II dose-ranging trial (study 2330/PALO-00-01).

The choice of the 15% non-inferiority margin set up in all three pivotal trials was chosen, based on the results of a meta-analysis of controlled phase II studies with historical placebo and active comparator in CINV.

Corticosteroids are the most commonly used drugs for the prevention of delayed emesis. Data on the concomitant use of corticosteroids were limited. No patients in study PALO-99-03 received corticosteroids, only 5% of the patients received this medication in study PALO-99-04 (after a protocol amendment) and only in study PALO-99-05, where 67% of patients received dexamethasone, corticosteroid use was permitted at the investigator discretion. It would have been more appropriate if use of corticosteroids had been introduced earlier, in all trials, as the evidence from many published studies and guidelines suggests that the addition of a corticosteroids to 5-HT_3 antagonists significantly improves antiemetic efficacy with each of the agents [31, 35, 36]. The SPCs of both ondansetron and dolasetron recommend the concurrent use of steroids to increase efficacy. Published data have led most investigators to advise that a corticosteroid should be added whenever the emetic source is thought to warrant a serotonin antagonist, unless a clearly documented reason for not using a corticosteroid in that patient has been demonstrated [6]. Therefore, the SPC of palonosetron mentions that the efficacy of palonosetron in the prevention of nausea and vomiting induced by highly emetogenic chemotherapy may be enhanced by the addition of a corticosteroid administered prior to chemotherapy (see section 4.2).

Whilst the efficacy of palonosetron in the treatment of acute onset nausea and vomiting has been demonstrated, the data provided for the demonstration of the efficacy of palonosetron in the treatment of delayed nausea and vomiting have been discussed.

The efficacy data collected between 24 and 120 hours in the pivotal trials were in accordance with the data observed on study day 1 and showed a sustained efficacy of palonosetron. However, these results were analysed as secondary endpoints and therefore were considered with caution.
Moreover, the choice of comparator and the treatment schedule used for the comparators did not allow assessment of the effectiveness of palonosetron in the prevention of delayed CINV. Ondansetron and dolasetron were under administered in inadequate doses for treatment of delayed emesis since patients received only one single dose before chemotherapy, which is not in line with the recommendations for the individual drugs. Data on the effect of a single dose of these comparators in preventing delayed nausea and vomiting are limited. Moreover, comparators have shorter half lives (4 and 7.3 hours respectively for ondansetron and dolasetron) than palonosetron (40 hours) and therefore comparative efficacy should not be mistaken by comparison of half lives.

In the other hand, the neuropharmacologic mechanism of nausea and vomiting of late onset is poorly defined. Risk factors for delayed emesis are mainly related to control of early emesis and to the chemotherapeutic agent used. In highly emetogenic CINV the emesis has multiphasic time courses whereas, in moderately emetogenic settings, emesis is described as a continuous phenomenon lasting for days after chemotherapy and fading off in days 4 to 5 [3, 37].
Therefore, palonosteron has been recommended for the prevention of acute nausea and vomiting associated with highly emetogenic cancer chemotherapy, and for the prevention of nausea and vomiting associated with moderately emetogenic cancer chemotherapy (see SPC section 4.1).

An open-label repeat cycle trial using the higher dose of 0.75 mg (study PALO-99-06) was performed to assess the efficacy of palonosetron over repeat chemotherapy cycles. No differences were noted regarding the efficacy profile of palonosetron compared with the other 5-HT_3 antagonists. However, from the efficacy point of view, this study was considered of limited value, since the efficacy of palonosetron was not assessed under controlled conditions, the higher dose investigated in the pivotal studies was used (0.75 mg) rather than the recommended dose (0.25 mg), and the number of patients with three or more courses was limited. The overall safety was maintained during all cycles (see SPC section 5.1).
Moreover, palonosetron was shown to have a half-life considerably longer than that of other 5-HT_3 antagonists (approximately 40 hours compared to 3-12 hours) and accumulation occurred with daily doses of 0.25 mg (see PK, study PALO-02-12 and 2330). While high exposure were shown to be safe

and well tolerated, repeated dosing of palonosetron within a seven day interval is not recommended (see SPC section 4.2).

No formal study was conducted in children. The final study report of an ongoing phase III trial (PALO-99-07) to assess the safety, pharmacokinetics and efficacy of single i.v. 3 or 10 µg/kg dose of palonosetron in paediatric patients receiving moderately or highly emetogenic chemotherapy should be submitted following completion of the study. The use of palonosetron in patients under 18 years of age is not recommended until further data become available (see SPC section 4.2 and post-approval commitment on study PALO-99-07).

Clinical safety

Clinical safety data were collected from 18 clinical studies conducted with i.v. and oral palonosetron administered to healthy volunteers, special populations, patients with CINV and PONV. Only the 16 single-dose studies were included in an Integrated Safety Database. The two non-integrated studies included a Phase I crossover interaction study (PALO-99-34) in which healthy volunteers received two single i.v. doses of palonosetron, and a phase III uncontrolled repeat-cycle study (PALO-99-06), which involved patients with CINV, previously enrolled in a phase III study.

Adverse events (AE) occurring during the 14-day study period in the 16 integrated studies were included in the analysis of adverse events. Deaths and serious adverse events occurring during the 30 days post-dose, as well as discontinuations due to AEs, were analysed for all 18 clinical studies, including the two non-integrated studies PALO-99-34 and PALO-99-06.

- Patient exposure

A total of 3125 individuals were observed in clinical trials and included in the integrated safety data base studies. Of these, 2348 received palonosetron, while 410 and 194 received ondansetron 32 mg and dolasetron 100 mg, respectively, and 173 received placebo. Of the 2348 patients receiving palonosetron, 895 patients in pivotal phase III studies enrolled in an open label continuation study and received more than one dose.
Of the 3125 individuals, 2348 received a single dose (1838 i.v. and 510 oral) of palonosetron (<0.25 mg: 341; 0.25 mg: 841; 0.75 mg: 819; 347 > 0.75 mg: 347) and of these, 1545 were treated for CINV, 605 for PONV and 198 were healthy volunteers and special populations. The 0.25 mg and 0.75 mg doses represent 71% of palonosetron treated patients.
Of the palonosetron treated patients, 1374 (59%) were enrolled in the pivotal phase II/III studies. 1838 patients (78%) received i.v. doses, and 510 (22%) received palonosetron orally. Thirty-three percent of palnosetron patients received highly emetogenic chemotherapy and 32% received moderately emetogenic chemotherapy.
In the integrated Phase I, II and III studies, the mean age of palonosetron treated patients was 49.7 years and 18% patients were at least 65 years old. More females (64%) than males (36%) received palonosetron and the predominant race studied was Caucasian (67%). There were slightly higher numbers of females in the dolasetron group.

Safety in patients receiving repeat cycles of palonosetron was evaluated from study PALO-99-06, where 895 patients were treated with palonosetron 0.75 mg in at least one chemotherapy cycle. There were 446 patients receiving two cycles (50,1%), 220 receiving three (24,1%), 82 receiving four (9%), 46 receiving five (5,2%), 20 receiving six (2,2%), 14 receiving seven (1,6%), 7 receiving eight (0,8%) and 3 receiving nine cycles (0,3%), for an overall total of 1726 single treatment cycles. The mean number of cycles was of 1,9. Corticosteroids were used in approximately 20% of the subjects in every cycle.

- Adverse events

A total of 1693 of 2348 (72%) palonosetron treated patients reported at least one AE.

Overall, gastrointestinal symptoms were most common in all treatment groups, regardless of relationship to study drug, and were reported in 30% of patients treated with palonosetron and dolasetron (708/2348 and 58/194, respectively), in 25% of patients taking placebo (43/173) and in 20% of patients treated with ondansetron (83/410). Gastrointestinal symptoms ranged from 27 % at the 0.25 mg dose to 39% in the over 0.75mg dose. Constipation was most commonly reported, followed by diarrhoea and abdominal pain.
Nervous system AEs were reported for 27% of palonosetron treated patients (632/2348), 25% of placebo patients (44/173) and 29% of patients taking the active comparators (173/604). Headache was most commonly reported, followed by insomnia.
Blood and lymphatic system disorders were reported for 14% of the palonosetron treated patients (317/2348) and more frequently for the patients treated with the active comparators (22% for ondansetron, 25% for dolasetron). Metabolic disorders were also more commonly reported in the active comparator groups (ondansetron 15%, dolasetron 12%) than in the palonosetron groups (11%).

From pivotal II/III studies, the most commonly reported AEs were gastrointestinal in 28% of patients (including constipation in 11%, diarrhoea in 6% and abdominal pain in 4% of patients), nervous system AEs in 25% of patients (including headache in 19% of patients), general AEs in 24% of patients (including fatigue, pyrexia, asthenia and weakness) and blood and lymphatic events (21%).
The most frequently events considered related to study drug administration were: headache, constipation, diarrhoea, and dizziness. For constipation, within palonosetron treatment groups, incidences ranged from 5% at the 0.25 mg dose to 7% at the 0.75 mg dose, while the comparator incidences ranged from 2% to 6% in the ondansetron and dolasetron groups, respectively. The incidence of diarrhoea was 1% in both palonosetron groups (0.25 mg and 0.75 mg) and 2% in the comparator groups. The incidence of headache was similar and ranged from 9% to 12% in the palonosetron groups (0.25 mg and 0.75 mg, respectively) and from 8% to 16% in the ondansetron and dolasetron patients, respectively. Dizziness occurred in 1% of patients treated with both doses of palonosetron, while its incidence was 2% for both active comparators.
All common (>1/100 to <1/10) and uncommon (>1/1 000 to <1/100) adverse events reported inpatients treated with palonosetron 0.25 mg are summarised in table 23.

Table 23: Common and uncommon adverse events reported in patients treated with palonosetron 0.25 mg in phase II/III studies by SOC.

System Organ Class	Common (> 1/100 to <1/10)	Uncommon (>1/1 000 to < 1/100)
Metabolism and nutrition disorders		Hyperkalaemia, metabolic disorders, hypocalcaemia, anorexia, hyperglycaemia, appetite decreased
Psychiatric disorders		Anxiety, euphoric mood
Nervous system disorders	Headache Dizziness	Somnolence, insomnia, paraesthesia, hypersomnia, peripheral sensory neuropathy
Eye disorders		Eye irritation, amblyopia
Ear and labyrinth disorders		Motion sickness, tinnitus
Cardiac disorders		Tachycardia, bradycardia, extrasystoles, myocardial ischaemia, sinus tachycardia, sinus arrhythmia, supraventricular extrasystoles
Vascular disorders		Hypotension, hypertension, vein discolouration, vein distended
Respiratory, thoracic and mediastinal disorders		Hiccups
Gastrointestinal disorders	Constipation Diarrhoea	Dyspepsia, abdominal pain, abdominal pain upper, dry mouth, flatulence
Hepato-biliary disorders		Hyperbilirubinaemia
Skin and subcutaneous tissue disorders		Dermatitis allergic, pruritic rash
Musculoskeletal and connective tissue disorders		Arthralgia
Renal and urinary disorders		Urinary retention, glycosuria
General disorders and administration site conditions		Asthenia, pyrexia, fatigue, feeling hot, influenza like illness

With regard to constipation, within palonosetron treatment groups, incidences ranged from 5% at the 0.25 mg dose to 7% at the 0.75 mg dose, while the comparator incidences ranged from 2% to 6% in the ondansetron and dolasetron groups, respectively. The incidence of diarrhoea was 1% in both palonosetron groups (0.25 mg and 0.75 mg) and 2% in the comparator groups.

The intensity of adverse events (as assessed by the investigator) of phase II/III studies, regardless of treatment relationship is summarised in table 23, by system organ class and treatment group.

Table 23: Intensity of AEs in pivotal phase II/III studies by SOC (number (%) of patients)

	Palonosetron (all doses) N = 1374			Active comparators (combined)[a] N = 604		
System organ class	**Mild (%)**	**Moderate (%)**	**Severe (%)**	**Mild (%)**	**Moderate (%)**	**Severe (%)**
No AEs	360 (26%)			172 (28%)		
Any AEs	44	25	5	41	26	4
Blood	8	10	3	7	13	3
Cardiac	5	1	<1	4	1	<1
Ear	1	<1	0	1	<1	0
Endocrine	<1	<1	0	0	0	0
Eye	1	<1	<1	<1	<1	0
Gastrointestinal	18	9	2	15	7	1
General	13	9	2	15	5	1
Hepatic	1	<1	0	1	<1	<1
Immune	<1	<1	0	0	<1	0
Infection	3	3	1	3	3	<1
Injury/Poison	<1	<1	<1	<1	0	0
Investigational	7	6	1	6	6	1
Metabolic	9	5	1	10	3	1
Musculoskeletal	3	3	<1	4	2	<1
Neoplasm	<1	<1	<1	<1	0	<1
Nervous	18	6	1	22	6	1
Psychiatric	2	1	<1	1	<1	0
Renal	2	1	<1	2	<1	<1
Reproductive	1	<1	<1	<1	<1	0
Respiratory	4	2	1	3	<1	<1
Skin	4	2	<1	4	2	1
Surgical	1	1	0	1	<1	0
Vascular	2	2	<1	3	1	1

[a] Ondansetron 32 mg (n = 410), dolasetron 100 mg (n = 194)

Dose repeat study

The incidence of AEs by patients was 74.6%, and the incidence of adverse drug reactions was 23.5%. Overall, 2737 AEs were reported in 1113 cycles (64,5%) of 665 patients, and the incidence of study drug related AEs reported by 209 subjects was 16.1% (429 AEs in 278 cycles).

The most frequently reported AEs were headache (total cycles 14.0%), lymphopenia, leucopenia and neutropenia (total cycles 10.8%, 8.0% and 6.2%, respectively). Of all AEs, 429 in 278 cycles (16,1%), reported by 209 patients were considered treatment related; headache and constipation were the most frequently reported drug-related adverse events (7.7% and 3.4%, respectively).

There was no relevant difference between cycles in terms of the rate of drug-related AEs. The frequency of related AEs in the first three cycles was: 20,4%, 13,3% and 10,4% in cycle 1, 2 or 3 respectively.

Cardiac safety

Due to the fact that 5-HT_3 receptor antagonists have shown to influence cardiac ion channel function, resulting in potential clinical effects on the depolarisation (QRS duration) and repolarisation (QTc duration) phases associated with varying degrees of ECG changes, an extensive programme was performed on palonosetron to assess its potential cardiac conduction effects.

ECG data from the integrated selected Phase I, II and III palonosetron clinical studies (PALO-01-18) revealed that palonosetron and active comparators had no clinically relevant effects on heart rate, PR, QRS or QT intervals and did not cause any new ECG morphological changes (conduction blocks, ST-T waves and myocardial infarction patterns). The ECG results of the studies that were not included in the integrated ECG analysis (Phase II PONV [2500 and 2502], early terminated CINV study 2120, and late Phase I studies PALO-99-39, PALO-99-35 and PALO-99-51) were not submitted as they were either not evaluated by the central facility or were considered outside the scope of the evaluation.

Holter monitoring was collected in 193 patients. Palonosetron did not produce any clinically relevant supraventricular or ventricular arrhythmias, including Torsades de Pointes, or atrio-ventricular conduction defects.

No studies on the effects on the ability to drive and use machines have been performed.

- Serious Adverse Events (SAE)

In the 16 integrated studies, a total of 135 patients treated with palonosetron (6%), 21 in ondansetron group (5%), 9 treated with dolasetron (5%) and five with placebo (3%), reported SAEs considered or not related to palonosetron. The most common SAEs reported by the patients treated with palonosetron, were general (n = 30), and gastrointestinal (n = 30), metabolic (n = 29), infection (n = 27) and blood (n = 24) disorders. For each of these SAEs, the incidence was 1%. There were no differences between dose groups (range 5% to 8%) or comparators (5%).

Eight patients had SAEs which were considered possibly or probably related to palonosetron. Six of these were involved in the single dose studies. Two cases of constipation have been reported (Study 2502 and PALO-99-35). No SAEs were reported as probably or possibly related to active comparators or placebo.

- Deaths

Overall, 35 patients died, including 31 who had received palonosetron (1%) and four who had received ondansetron as active comparator (1%). There were no deaths among dolasetron or placebo patients. No deaths were considered to be related to palonosetron. Among the 31 patients treated with palonosetron who died, 28 (1%, 28/2348) were enrolled in studies included in the Integrated Safety Database and three (< 1%, 3/895) were enrolled in the non-integrated repeat-cycle study PALO-99-06. Deaths were reported during phase II and III studies. Two of the deaths occurred in phase II studies in patients treated for PONV and were caused by pulmonary embolism and ovarian cancer. The other 29 deaths (13 in phase II and 16 in phase III) occurred in patients receiving moderately or highly emetogenic chemotherapy for cancer and were associated with conditions not unexpected in this population.

- Laboratory findings

Changes in haematology parameters were no different between treatment groups, and were attributed to chemotherapy. The most common haematology-related AEs reported for the palonosetron patients in phase II/III studies, regardless of dose, were lymphopenia (9%), leucopenia NOS (9%), neutropenia (5%), anaemia NOS (3%) and thrombocytopenia (2%).
Transient alteration in hepatic enzymes (elevated transaminases) was observed across all treatment groups and was considered principally related to the type of chemotherapy.
Hypokalaemia (2%) and hyperkalaemia (1%) were observed.

- Safety in special populations

The differences in incidence of AEs observed in the phase II/III trials, between age, gender or race subgroups were not clinically significant.
No specific safety data in patients with renal or hepatic impairment enrolled in phase II/III studies were provided. Further to the request of the CHMP, the applicant stressed that in Phase II studies, patients with renal or hepatic impairment were excluded from enrolment, and in phase III studies, no special inclusion/exclusion criteria applied for enrolment of patients with renal or hepatic disorders, meaning that these patients could have been enrolled on the basis of the investigator's medical opinion. The information relevant to the renal and hepatic impairment was systematically recorded in the CRF and presented to the CHMP.

- Safety related to drug-drug interactions and other interactions

Study PALO-99-34 has been performed to investigate the pharmacokinetic interaction between palonosetron and metoclopramide. This study was not included in the Integrated Safety Database.
No new safety concerns were raised form this study and the safety data were consistent with the safety profile described in the integrated phase I, II and III studies.

The cardiovascular safety of patients exposed to doxorubicin at doses ranging from 1 mg to > 550 mg (cumulative dose within 12 months) during the pivotal phase III studies, were also assessed. This study did not raise any safety concerns.

- Discontinuation due to AES

In integrated phase I, II, III studies the rate of early discontinuation due to adverse events, regardless of relation to the study medication, was 0.8% in the palonosetron group, with a similar incidence in the ondansetron group (<1%) and between different doses of palonosetron. No patient discontinued in the dolasetron group. Among AEs reported by the patients treated with palonosetron who discontinued (n=100), eight were considered related to the medication.
Of the 18 palonosetron treated patients who discontinued due to AEs, 14 had an outcome of death. A review of the narratives of the 14 cases showed that most of the patients were elderly and had concurrent illness or symptoms which suggested that palonosetron was not causing or contributing to their deaths.

- Post marketing experience

Palonosetron (US brand: Aloxi®) has been on the market in the US since 15 September 2003. Since the introduction of palonosetron to the US market, 3 quarterly safety reports have been produced. In the period from 15 September 2003 to 24 April 2004 (the data lock point of the third quarterly report) approximately 285 000 units of palonosetron have been sold.
The applicant has provided the CHMP with a safety update based on marketing in the United States and with copies of the first three quarterly adverse drug experience reports for the FDA.

Of the 52 adverse drug reactions reported in that period, 51 are spontaneous post-marketing cases, and 1 was received from a clinical study. Nine cases were reported as serious (6 were considered serious and unexpected and 3 serious expected) and the remaining 43 were reported as non-serious (24 non-serious unexpected and 19 non-serious expected).
In the FDA letter of approval dated 25 July 2003, it was also requested that an expedited 15-day report be provided for any of the following adverse reactions:
- all spontaneous reports of constipation requiring hospitalisation or emergency room visit,
- all spontaneous reports of possible complications of constipation such as obstruction, perforation, intestinal ulceration, toxic megacolon, ileus or impaction resulting in hospitalisation or emergency room visit,
- all spontaneous reports of any cardiovascular event.
Of the 52 adverse drug reactions, 12 were reported as expedited 15-day reports and 6 of these were serious unexpected cases; 4 of these 6 cases were hypersensitivity reactions and 2 were reports of convulsions (one in a patient with carcinomatous meningitis and brain metastases, and the other one a grand mal seizure in a patient with documented cerebral infarcts).
Expected cases included headache with bradycardia, influenza like illness and 4 non-serious unexpected cases of hypersensitivity (<1/10,000) associated with hypotension, palpitations, and tachycardia.

The majority of the non-expedited unexpected reports included hypersensitivity reactions and injection site reactions (burning, induration, discomfort and pain).

- Discussion on clinical safety

The safety profile of palonosetron was similar to that of other 5-HT_3 receptor antagonists. The most common adverse events included headache, constipation, fever, abdominal pain, diarrhoea, pruritus, pain, asthenia and insomnia.

In the integrated safety database studies, the incidence of adverse events was comparable between the palonosetron (72%), ondansetron (69%) dolasetron (77%) and placebo (61%) groups, as well as among patients receiving different doses of palonosetron. The most common AEs reported in all treatment groups, regardless of relationship with study medication, were the gastrointestinal AEs, followed by nervous system AEs.
No case of overdose has been reported. In the unlikely event of overdose, patients should be monitored with supportive care (see SPC section 4.9).

The adverse events profile of a single dose of palonosetron in a unique cycle, was similar to ondansetron and dolasetron, according to the safety data obtained from controlled clinical trials. The most frequently adverse events related to palonosetron in pivotal phase II/III studies were headache, constipation, dizziness and diarrhoea occurring in 11%, 6%, 1% and 1% of patients, respectively.
Constipation as an adverse effect could be a class effect of 5-HT3 antagonists on bowel smooth muscle. The applicant was requested to investigate whether there was any evidence of drug related effects on bowel transit times. A review of the cases of constipation has shown that the incidence of constipation was similar to that seen with other drugs of the same class used as comparators. Patients with a history of constipation or signs of subactute intestinal obstruction should be monitored following the administration of palonosetron (see SPC section 4.4).

In all controlled studies, the control medication was given as a single dose and for a single cycle, which does not reflect the established use conditions, where antiemetics are usually given for repeat cycles.
The profile of AEs from one non-controlled repeat cycle study was consistent with the data obtained from single dose studies. However, this was the only repeat dose study and only 155 patients (≤ 9%) experienced four or more cycles. In addition, the dose used in this trial (0,75mg) was higher than the dose claimed for the marketing authorisation (0,25mg).

No dose-response effect on the ECG QTc interval has been shown with palonosetron.
However, considering that 5-HT_3 receptor antagonists have shown to influence cardiac ion channel function, resulting in potential clinical effects on the depolarisation (QRS duration) and repolarisation (QTc duration) phases associated with varying degrees of ECG changes, a warning has been included in section 4.4 of the SPC for patients with prolonged QT or receiving concomitantly, medicinal products that cause prolongation of the QT.

There was no evidence of an effect of age, gender or race on the palonosetron safety profile. Due to the small number of patients with various degrees of renal and hepatic impairment enrolled in each study, it was not possible to draw any conclusions in terms of incidence or type of adverse event in these populations. Based on the available data, frequency and type of adverse event were similar to those seen in the group as a whole.

In the absence of data on the effects of palonosetron on the ability to drive and use machines, since palonosetron may induce dizziness, somnolence or fatigue, the CHMP recommends that patients should be cautioned when driving or operating machines (see SPC section 4.7).

Post marketing data did not raise any new serious safety concerns. Very rare cases of hypersensitivity reactions and injection site reactions were reported and were included in the SPC. Hypersensitivity to the active substance or to any of the excipients of the formulation of the finished product is a contraindication of the use of the medicinal product (see SPC section 4.3).

5. Overall conclusions, benefit/risk assessment and recommendation

Quality

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. There are no unresolved quality issues, which have a negative impact on the Benefit Risk balance of the product.

Non-clinical pharmacology and toxicology

Palonosetron hydrochloride is a potent and selective 5-HT_3 receptor antagonist. In *in vivo* studies conducted in the rat, dog and ferret, palonosetron ameliorated the emetic effects of cisplatin, dacarbazine, actinomycin-D and mechlorethamine. Cardiovascular safety investigations did not show any other effects than the known class effects of 5-HT_3 antagonists on I_{Kr} and I_{Na} currents and action potentials, but at very high palonosetron concentrations. Safety pharmacology studies raised little concern about the effects of palonosetron on respiratory, GI, renal, central and autonomic system functions at therapeutic doses. The administration of palonosetron alone or in combination with chemotherapeutic agents affected neither the course of tumor pathology nor the anti-tumor effects of the drugs. However, the non-clinical pharmacodynamic drug interaction studies did not sufficiently address possible clinically-relevant adverse drug interactions.
Non-clinical pharmacokinetics studies showed that palonosetron was rapidly absorbed, with a low bioavailability, suggesting extensive pre-systemic metabolism and that it was extensively distributed. Protein binding was approximately 48 % in rat and 66 % in dog plasma. Although a range of metabolites was identified from animal oral studies, many were not relevant to the clinical situation, being products of first-pass metabolisms. In humans, after i.v. administration, palonosetron remains the primary plasma and urine component. Two major human metabolites, M4 and M9, have been identified. Elimination of palonosetron from tissues was parallel to plasma clearance, except in the eye. The results from genetic toxicology investigations showed that palonosetron was not genotoxic.
Two long-term studies assessed the carcinogenicity of palonosetron administered by oral gavage in rat and mouse. Increase of liver weights, keratoacanthoma were found in rats and proliferative lesions in the pancreas and pituitary were observed in males. These findings were not relevant to human and were considered acceptable. The reproductive and developmental studies conducted were appropriate and the NOAELs were high enough to allow a reasonable assumption of safety in human.

Efficacy

In double-blind randomised controlled trials, a single i.v. dose of palonosetron 0.25 mg was effective in the prevention of nausea and vomiting with moderately emetogenic chemotherapy and in the control of acute nausea and vomiting with highly emetogenic chemotherapy.
A single dose of ondansetron 32 mg or dolasetron 100 mg were used as comparators. In terms of efficacy, palonosetron was non-inferior to the comparators in the acute phase of emesis both in moderately and highly emetogenic setting, independently of whether patients had been pre-treated or not. However, no data on the mean number of prior cycles and the response to prior antiemetic therapy were provided. These studies were not designed to show an effect in late onset nausea and vomiting, and there were limitations for the interpretation of the efficacy after day 1 due to a non optimal use of comparators. However, considering the neuropharmacology of chemotherapy-induced emesis, palonosetron is indicated for the prevention of acute nausea and vomiting associated with highly emetogenic cancer chemotherapy, and for the prevention of nausea and vomiting associated with highly emetogenic cancer chemotherapy.

An open label repeat cycle trial using a higher dose (0.75 mg) of palonosetron has been performed, however this study was of limited value since comparative efficacy was not assessed.
Moreover, palonosetron has a half-life considerably longer than that of other 5-HT_3 antagonists and accumulation of palonosetron occurred with daily doses of 0.25 mg. Repeated dosing of palonosetron within seven day interval is therefore not recommended.

Data on the concomitant use of corticosteroids were limited, as only in study PALO-99-05 its use was permitted at the investigator discretion. No patients in study PALO-99-03 received corticosteroids and only 5% of the patients received this medication in study PALO-99-04, after an amendment to the protocol. However, the evidence from many published studies and guidelines suggests that 5-HT_3 receptor antagonists administered in combination with corticosteroids afford best protection from symptoms of acute and delayed emesis. The efficacy of palonosetron in the prevention of nausea and vomiting induced by highly emetogenic chemotherapy may be enhanced by the addition of a corticosteroid administered prior to chemotherapy.

No efficacy data were available in paediatric patients at the time of the assessment. The use of palonosetron in patients under 18 years of age is not recommended until further data become available (see SPC section 4.2 and post-approval commitments on study PALO-99-07).

Safety

The safety of palonosetron was similar to the currently available 5-HT_3 receptor antagonists. The most common adverse events included headache, constipation, fever, abdominal pain, diarrhoea, pruritus, pain, asthenia and insomnia. Preclinical studies indicated a potential to cause prolongation of the QT interval. At all dose levels tested in clinical studies, palonosetron did not induce clinically relevant prolongation of the QTc interval. However, as for other 5-HT_3 antagonists, caution should be exercised in the concomitant use of palonosetron with medicinal products that increase the QT interval or in patients who have or are likely to develop prolongation of the QT interval.

Benefit/risk assessment

From a clinical perspective, the benefit risk relationship was considered favourable. With a safety profile that was similar to the currently available 5-HT_3 receptor antagonists, palonosetron was effective in the control of nausea and vomiting with moderately emetogenic chemotherapy and of acute nausea and vomiting with highly emetogenic chemotherapy. The design of a repeat cycle trial did not allow to conclude on the efficacy of palonosetron over repeated chemotherapy cycles.
Repeated dosing of palonosetron within a seven-day interval was not recommended considering its important half-life and the risk of accumulation of palonosetron with daily doses of 0.25 mg.

The efficacy of palonostetron in the prevention of nausea and vomiting induced by highly emetogenic chemotherapy may be enhanced by the addition of a corticosteroid administered prior to chemotherapy.

The indication: “Prevention of acute nausea and vomiting associated with highly emetogenic cancer chemotherapy and prevention of nausea and vomiting associated with moderately emetogenic cancer chemotherapy” was readjusted compared with the initial proposed wording, in order to exclude “Prevention of nausea and vomiting associated with initial and repeat course of emetogenic cancer chemotherapy” from the scope of the indication, since the pivotal studies were not designed to show an effect over repeated chemotherapy cycles.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered that the benefit/risk ratio of Aloxi (palonosetron) in: the prevention of acute nausea and vomiting associated with highly emetogenic cancer chemotherapy and the prevention of nausea and vomiting associated with moderately emetogenic cancer chemotherapy was favourable and therefore recommended the granting of the marketing authorisation.

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