SCIENTIFIC DISCUSSION

1. Introduction

This is a complete and independent application for Marketing Authorisation in accordance with Article 8.3(i) of Directive 2001/83/EC as amended, with a new active substance Gadofosveset trisodium.

X ray angiography (XRA) is widely used in vascular disease diagnosis because of its accuracy relative to other less invasive alternatives. However, a certain amount of morbidity and even mortality is associated with XRA in such patients. This morbidity includes vascular injury (conservatively estimated at 0.4%), nephrotoxicity leading to clinically significant nephropathy (1.6%), nerve damage, stroke, limb loss and death. Other disadvantages of catheter angiography include the need for an arterial puncture and exposure of the patient to ionizing radiation. An estimated 4 million patients per year undergo invasive, catheter-based XRA in the EU.

Magnetic Resonance Imaging (MRI) is based on the principle of nuclear magnetic resonance. MRI is a tomographic imaging technique that can acquire images in virtually any orientation. Images are derived from signals produced by protons (hydrogen nuclei), which are present in abundance because the human body consists mainly of water. The proton behaves like a small magnet when placed in a magnetic field: it aligns with the field and precesses with a given frequency that depends on field strength. Protons will align parallel and antiparallel to the direction of the primary field, with a small excess of parallel protons that gives rise to a net magnetisation vector. This net vector can be altered, i.e., rotated-by application of a secondary temporary radiofrequency pulse. Once this pulse is discontinued, the magnetisation vector starts to recover back to its former position, releasing signal in the form of radiowaves. This relaxation of net vector is attributable to two distinct but simultaneous processes, referred to as the longitudinal (T1) and the transverse (T2) relaxations.

In the past years, magnetic resonance angiography (MRA) has been recognised as a powerful tool in the evaluation of vascular disease in a majority of anatomic territories. Various studies on the direct comparison of MRA versus contrast X ray angiography have demonstrated the high potential of the lesser-invasive MR technique and its advantage concerning the three-dimensional approach to the vessel and the pathology. It may be applied with or without contrast media depending on the area of investigation, on the physiological properties of the interesting vessels, and mainly on the clinical question. Non-enhanced MRA is still advocated in situations in which motion of the vessels is limited to minor pulsation. This allows for longer imaging periods, correlated with beneficial higher resolution of the resulting images. In other vascular territories, vessels are affected by secondary motion such as breathing or peristalsis, and related artefacts prevent the application of longer-lasting MRA measurements. Other inherent problems of native techniques, such as saturation effects and phase wrapping MRA, are well known and further support the introduction of contrast-media-enhanced vascular evaluations.

Vasovist is a formulation of a stable gadolinium diethylenetriaminepentaacetic acid (GdDTPA) chelate substituted with a diphenylcyclohexylphosphate group (gadofosveset trisodium) for contrast-enhanced magnetic resonance angiography in patients with suspected or known abdominal or limb vascular disease. Gadolinium (Gd) is a rare earth metal that causes signal enhancement on MRI images by interacting with water molecules and shortening the T1 of the water molecules that interact with the Gd atom. For safety reasons Gd is administered only in chelated form because it is a heavy metal. Vasovist is highly and reversibly bound to albumin which enhances the paramagnetic effectiveness of gadolinium and extends the vascular lifetime of the agent thus allowing long vascular imaging time and high spatial resolution. In the text both MS-325 and Gadofosveset are used[1].

[1] both referring to the active substance.

2. Quality aspects

Introduction

Gadofosveset trisodium is a magnetic resonance imaging agent for intravenous administration composed of a 0.25 M aqueous solution of Gadofosveset trisodium (Gadofosveset) and Fosveset as free ligand. It should be administered as a single bolus injection, manually, or by power injection over a period of time up to 30 seconds followed by a 25-30 ml normal saline flush.

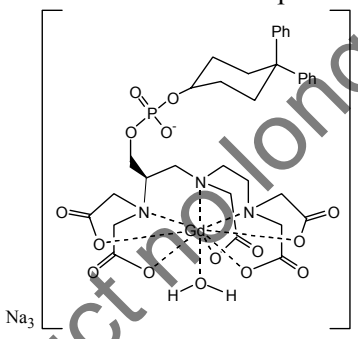
Dosage: Adults: 0,12 ml/kg body weight (equivalent to 0,03 mmol/kg).
The product is presented in 3 single dose forms: 10 mL fill in a 10 mL vial (10/10 mL), 15 mL fill in a 20 mL vial (15/20 mL) and a 20 mL fill in a 20 mL vial (20/20 mL).

The primary container consists of a single use Type I glass vials with bromobutyl stoppers

Excipients are water for injection, sodium hydroxide, hydrochloric acid and fosveset

Active Substance

Gadofosveset trisodium is a white to slight yellow non-crystalline powder very soluble in water and hygroscopic in nature. It decomposes at a temperature above 275°C.
The molecule contains 2 chiral centres and exists as an equilibrium mixture of 2 interconvertible diastereoisomers (Isomers A and B). The conversion rate is pH and temperature dependent.



Gadofosveset Trisodium

- **Manufacture**

The chemical synthesis of this new active substance takes place in 6 steps.
Purification by reverse phase chromatography then occurs. The solution is concentrated by wiped-film evaporation and the product ultra-filtered. Gadofosveset Trisodium is isolated by spray-drying.

The manufacturing process has been adequately described. Critical parameters have been identified and adequate in-process controls included.
Specifications for starting materials, reagents, catalysts and solvents have been provided. Adequate control of critical steps and intermediates have been presented.
Structure elucidation has been performed by elemental analysis, infrared spectroscopy, ultraviolet spectroscopy, optical rotation, and mass spectrometry.

Physico-chemical parameters such as polymorphism, solubility, particle size have been studied. X-ray analysis demonstrated that the substance is an amorphous powder. Polymorphism and particle size are not critical since the finished product is an aqueous solution.

Impurities have been extensively described, classified as process related impurities and possible degradation products, and qualified with reference to toxicological studies.

Residual solvents and residual metals have been satisfactorily controlled in the active substance. Limits are in accordance with ICH requirements.

- **Specification**

The specification of the active substance is appropriate and includes appearance, identification tests, purity (related substances, enantiomeric excess, isomeric ratio, residual solvents), assay of gadosfoveset trisodium, microbiological quality. Analytical methods have been described and validated.
Batch analyses confirm satisfactory compliance and uniformity with the proposed specifications.
References standards used for the active substance have been fully characterised.

- **Stability**

Stability studies have been conducted under ICH conditions (up to 36 months at 25°C/60% RH, 1 batch stored 12 months at 30°C/65% RH and 6 months at 40°C/75% RH) where the active substance is stored in a double Low Density Polyethylene bag, placed in High Density Polyethylene drum closed by a HDPE cap representative of the marketing pack.
Additional studies demonstrate that the substance is not sensitive to light but is hygroscopic.
Based on the stability data, it can be concluded that the active substance is very stable and the results support the re-test period of 3 years provided the substance is protected from moisture.
A satisfactory post-approval stability protocol has been presented (first 3 commercial batches to be tested, then 1 batch per year).

Other ingredients

All formulation excipients and nitrogen process aid comply with Ph. Eur. specifications except for Fosveset.
Comprehensive information on Fosveset has been provided, including full details of synthesis, specifications, analytical methods and stability data. Its synthesis is almost identical as the active substance synthesis except the last step. A re-test period of 3 years with no special precaution of storage can be granted.
The applicant has confirmed that one ingredient (L-serine methylester hydrochloride) was from enzymatic origin, but has no significant risk in the context of TSE.

Medicinal Product

- **Pharmaceutical Development**

This injectable formulation contains gadosfoveset trisodium (as the active substance), fosveset (as ligand, and is a new excipient), sodium hydroxide and hydrochloric acid (used for the pH adjustement) and water for injection (as the solvent). Compatibility with all the excipients has been demonstrated.
Since the product is a solution, and the active substance is very soluble, particle size does not affect bioavailability and/or stability. Polymorphism does not occur and is therefore not critical.
Formulation studies showed that 250 mM of the active substance, with 0.1% of fosveset and a pH close to physiological pH would be a suitable and stable formulation, with an appropriate concentration and viscosity for a small volume injection at the anticipated human dose. No overages need to be included.
The manufacturing process development has been satisfactorily described.
This sterile solution is packaged in clear type I glass vials, sealed with bromobutyl rubber stoppers suitable for parenteral product.

- **Manufacture of the Product**

The manufacture of the finished product is sufficiently detailed. The process can be summarised as follow : dissolution of the active substance in water, addition of a solution of fosveset, pH adjustment and specific gravity adjustments, sterilising pre-filtration of the solution, filling the vials and terminal sterilisation by heat moisture is in accordance with PhEur. requirements. This should guarantee the sterility of the solution.
In-process controls such as pH, assay of the manufacturing steps are sufficiently described and adequate.
Standard method of manufacture is used and complies with Ph. Eur. requirements. Validation data for the manufacturing process are presented in the dossier and considered sufficient.

Specifications and batch analysis are provided for each excipient.

All formulation excipients and nitrogen process aid comply with Ph. Eur. specifications except for Fosveset. A satisfactory in-house specification has been established for this new excipient.

- **Product Specification**

Release and end-of-shelf life specifications including appearance, identification, assay of gadosfosveset, isomer ratio, related substances, pH, sterility, endotoxins, particles, osmolarity, viscosity, relative density and volume are provided, and are appropriate for an injectable solution.
Analytical methods have been described and validated.

Batch analysis of 20 batches confirms consistency and uniformity of manufacturing and show that the process is under control. Impurities limits have been satisfactorily justified.

- **Stability of the Product**

Stability studies on the finished product manufactured at the proposed commercial site have been performed under ICH conditions (up to 36 months at 25°C/60% RH, and 6 months at 40°C/75% RH) for three batches of each packaging size. Batches were stored upright and inverted in the proposed marketing packs. Matrixing has been applied to the protocol.
Samples were tested for all parameters that could evolve during storage (all parameters mentioned in the product specifications and additional tests such as enantiomeric excess, container closure integrity, free ligand, heavy metals, relaxivity).
No specific trend was observed and the product remains stable throughout the testing period.
Separate studies have been carried out to complete the stability data (freeze/thaw, photostability in accordance with ICH guideline, stability after autoclaving and under elevated temperatures. Data showed that the product is stable after freeze/thaw cycles, photosensitive and stable under elevated temperatures except for the pH increase.
As a conclusion from the stability studies, the results support the proposed shelf-life and storage conditions as defined in the SPC.
A satisfactory post-approval stability protocol has been agreed.

Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the drug substance and the drug product have been presented in a satisfactory manner. The results of tests carried out indicate satisfactory consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product shouls have a satisfactory and uniform performance in the clinic.
At the time of the CHMP opinion, there were a number of minor unresolved quality issues having no impact on the Benefit/Risk ratio of the product. The applicant gave a letter of undertaking and committed to resolve these as Follow Up Measures after the opinion, with an agreed timeframe.

3. Non-clinical aspects

Introduction

Most of the pharmacological tests and single dose pharmacokinetic studies were not strictly conducted according to GLP. Pivotal safety pharmacology studies were performed according to GLP. Studies on toxicology and toxicokinetics were performed according to GLP, with a few exceptions.

Pharmacology

Contrast agents for MRI shorten the T1 and T2 relaxation times of water protons. The influence on the relaxation rates such as the longitudinal relaxation rate (1/T1) or the change in relaxation rates (Δ1/T1) are used to describe MR contrast agent effects. The efficacy of a contrast agent is most commonly described by the term relaxivity (r_1 and r_2, respectively) which is the change in relaxation rates divided by concentration of the contrast agent. The higher the relaxivity, i.e. the more pronounced the effect on shortening of the relaxation times is, the more effective is the compound to reach a significant contrast enhancement for diagnostic purposes. Furthermore, the binding of a MR contrast agent to

macromolecules (e.g. serum albumin in the case of MS-325) leads to a higher relaxivity and increases contrast.

- Primary pharmacodynamics (*in vitro/in vivo*)

In vitro

To determine the binding sites of MS-325 on human serum albumin (HSA) the binding of the fluorescent probes dansylsarcosine (DS) and dansyl L-asparagine (DAsn) to HSA were evaluated in the presence of MS-325. DAsn binds to site I on subdomain IIA, while DS binds to site II on subdomain IIIA of HSA. MS-325 displaced DS with an inhibition constant (K_i) of 85 µM. MS-325 showed very weak displacement of DAsn (K_i = 1500 µM), indicating that MS-325 binds primarily to site II on subdomain IIIA of HSA. Gadofosveset exists as an equilibrium mixture (1.3-1.9:1) of two interconvertible diastereoisomers, denoted as Isomer A and Isomer B. Both isomers bind to the same site on MS-325 with similar affinity.

To determine the mechanism of action of MS-325, the binding of MS-325 to HSA solution was studied over a broad concentration range (0.06 - 50.3 mM) using an ultrafiltration assay. In a further study (concentration rate: 0.05 – 5.3 mM) the binding of MS-325 to HSA and in addition the longitudinal (1/T1) relaxation rates were measured. MS-325 was shown to bind to HSA in a concentration-related manner. At 0.05 mM and 0.06 mM, the lowest concentrations tested, the percentage bound to HSA was 88 % and 94 %, respectively. The degree of binding decreased with increasing MS-325 concentrations to 37 % at 5.3 mM and 28 % at 50.3 mM, the highest concentrations studied. The observed relaxivity in HSA was dependent upon the total MS-325 concentration and the magnetic field strength. The relaxivity r_1 was lower at higher magnetic field strength and decreased with increasing MS-325 concentrations. The relaxivity r_1 of MS-325 and of both isomers was similar and approximately 8 times that of a non-albumin binding agent (GdDTPA, r_1 = 5.1 $mM^{-1}s^{-1}$) at a concentration of 0.1 mM in 4.5 % HSA.

The binding of MS-325 to human plasma using the ultrafiltration assay was studied over a broad range of MS-325 concentrations (0.02 – 20 mM). GdDTPA was used as reference standard since it is known to have practically no protein binding. In addition in two studies the relaxivity at two magnetic field strengths (20 MHz = 0.47 T, 60 MHz = 1.41 T) was determined in the samples. At plasma concentrations up to 2 mM (most relevant to imaging) the fraction of MS-325 bound to the target tissue (plasma) was high (96 - 75 %). Larger relaxivities (r_1 and r_2) were determined in plasma for MS-325 than GdDTPA at clinically relevant concentrations. At higher concentrations of MS-325 the degree of binding to human plasma and the relaxivity decreased.

In another study the binding of MS-325 to different human plasma components (human serum albumin, α_1-acid glycoprotein, γ-globulins) was investigated. MS-325 bound exclusively to human serum albumin. In a further study the in vitro MRI signal intensity changes in human plasma for MS-325 and other commercially available contrast agents (Magnevist, ProHance, Omniscan) were investigated. The data showed that the T1 weighted MRI signal intensity for MS-325 in human plasma was greater than that obtained for the three commercially available contrast agents over the clinically relevant concentration range of 0.1 to 2 mM. The commercially available contrast agents required approximately five times higher concentration to achieve the same maximum signal intensity as MS-325.

The binding and the T1 relaxivity (r_1) of MS-325 were assessed in vitro in species-specific albumin or plasma of mice, rats, rabbits, dogs, pig, and nonhuman primates. Data showed high protein binding to albumin and plasma solution (66.9-96.8%) and a large increase in relaxivity r_1 in all species (22.6-46.7 $mM^{-1}s^{-1}$; buffer alone 6.6 $mM^{-1}s^{-1}$).

In vivo

In vivo studies with MS-325 in rats, rabbits, cynomolgus monkeys and baboons (doses ranging from 0.025 to 0.10 mmol/kg) demonstrated that the high plasma concentration resulted in a persistently high plasma 1/T1 and large increases in r_1. Two further studies in rabbits demonstrated the equivalence of different formulations and of different diastereoisomers of MS-325.

Two imaging studies were performed during the non-clinical development of MS-325. The first study (dose finding) utilized a standard MRA protocol to acquire images of rabbit hind-limb vessels at doses of 0.015, 0.025, 0.05 and 0.10 mmol/kg of MS-325. The results showed that the increase in the blood longitudinal relaxation rate raised the blood signal on MRI scans. Large vessel visualization was possible at all doses and small vessel visualization at doses greater than 0.015 mmol/kg. In addition, the increase in large and small vessel enhancement was modest when the dose was increased from 0.05 to 0.10 mmol/kg.

In another imaging study, the ability of MS-325 to image stenotic vessels was evaluated in a pig model of renal artery stenosis after administration of 0.05 mmol/kg. Dynamic (during or immediately after contrast agent administration) and steady-state MS-325-enhanced three-dimensional (3D) MRA images were compared against the standard clinical techniques of contrast XRA and pre-contrast two-dimensional time-of-flight MRA in the presence of gradually increasing stenosis. Results showed that pre-contrast time-of-flight MRA was able to detect only some stenotic vessels (6 out of 21). MS-325-enhanced dynamic 3D MRA was able to depict stenosis in all cases, and steady-state 3D MRA with MS-325 depicted stenosis in most cases (18 out of 21). A high degree of arterial detail with minimal enhancement of venous structures was observed. Arterial contrast - to - noise was good, with detectable left renal artery stenosis. In addition, small intercostal and vertebral arteries (ca. 2 mm in diameter) were visible. A good agreement in the measurement of the degree of stenosis determined by dynamic MS-325-enhanced 3D MRA and by contrast XRA was observed. MS-325 improved the ability of MRA to image diseased vessels, even in the case of severe stenosis.

- Safety pharmacology

The effect of MS-325 on vital organ systems was investigated in several *in vitro* and *in vivo* studies.

Central and peripheral nervous system
No CNS-effects were observed at i.v. bolus doses up to 0.3 mmol/kg in a series of different studies in mice and rats (including tests on general behavior, convulsive threshold, pain threshold, body temperature).

Cardio-respiratory system
MS-325 administered to anaesthetised male dogs did not affect heart rate, carotid blood flow or ECG. An *iv* infusion dose of 1 mmol/kg transiently decreased mean arterial pressure 1 to 3 min post-dosing. The maximum decrease of 15 % occurred at 1 min post-dosing and returned to pre-dosing baseline by 3 min post-dosing.

In another study in anaesthetised dogs, a bolus *iv* dose of 0.3 mmol/kg resulted in a transient and slight increase in cardiac output and stroke volume and a transient decrease in pulmonary vascular resistance, with the maximum changes occurring at 2.5 min post-dosing. At a dose of 1 mmol/kg, MS-325 caused a transient and slight decrease in mean aortic pressure, total peripheral resistance, and pulmonary vascular resistance, with the maximum change occurring at 2.5 min post-dosing. In all cases, values returned to their pre-dosing baseline within 10 min post-dosing. At all doses tested, MS-325 showed isolated signs of arrhythmias and of nonspecific T-wave abnormalities.

In rising dose cardiovascular safety pharmacology studies in monkeys MS-325 did not alter arterial pressure, heart rate, ECG rhythm and ECG time intervals at doses of ≤ 3 mmol/kg. At higher doses, 3 out of 4 monkeys had vomiting episodes, but recovered immediately afterwards.

The risk of QT prolongation was assessed in the HERG-potassium channel assay, in isolated guinea pig papillary muscle preparations and in ECG of conscious dogs and monkeys. No QT-prolongation-related effects were observed.

No effects on respiration rate and respiration volume were found in anaesthetised dogs administered with *iv* doses of MS-325 up to 1.0 mmol/kg.

Other systems and tissues
In toxicity studies in rats and monkeys, the kidney was identified as the main target organ. MS-325 did not impair human kidney proximal tubule cell viability or function at clinically relevant doses. In a renal function study in rats some alterations of renal function were observed at 1 mmol/kg; in monkey, doses up to 2 mmol/kg did not alter renal function.

MS-325 at doses up to 1 mmol/kg showed no effect on charcoal propulsion in the gastrointestinal tract of conscious rats.

Further studies were performed to assess the effects of MS-325 on autonomic nervous system and smooth muscle organs, on blood (hemolysis test, effects on the blood coagulation system, effects on rabbit platelet aggregation) and on metal ion concentrations in blood. MS-325 at concentrations up to 0.1 mmol/L showed no effect on acetylcholine- , histamine- or barium-induced contraction in the isolated guinea pig ileum, no hemolysis in rabbit erythrocytes and no effect on ADP- or collagen – induced platelet aggregation in rabbits. At doses up to 0.3 mmol/kg no effect on prothrombin time (PT) and activated partial thromboplastin time (APTT) in rats was observed, while 1 mmol/kg prolonged PT and APTT for 30 and 10 minutes, respectively. Furthermore, MS-325 at doses up to 1 mmol/kg showed no effect on rabbit serum calcium, magnesium or iron concentration.

- Pharmacodynamic drug interactions

A series of *in vitro* drug interaction studies were performed to assess the ability of MS-325 to displace radiolabelled digitoxin, propranolol, verapamil and warfarin from their binding sites on HSA. MS-325 at concentrations of 0.3 or 0.9 mM did not displace digitoxin, propranolol or verapamil. A significant increase of the unbound fraction of warfarin was observed. This effect could not be confirmed in a second *in vitro* study using the same study design.

Further studies were performed to investigate the potential effect of commonly used drugs (warfarin, ibuprofen, digitoxin, diazepam, ketoprofen, naproxen, diclofenac, piroxicam, phenprocoumon, at concentrations up to 100 times the known clinical steady-state plasma concentration) on MRI efficacy. A significant decrease in measured relaxation rate was only observed in the presence of naproxen and ibuprofen; this effect was considered to be of no clinical relevance, because medication with these drugs will most probably occur after the diagnostic session.

Pharmacokinetics

A validation of an inductively coupled plasma atomic emission spectrometry (ICP-AES) method for the concentration measurement of Gd (the magnetic resonance contrast enhancing component of MS-325), was performed in serum and aqueous samples. In addition, a high performance liquid chromatography, tandem mass spectrometric detection method and an inductively coupled plasma with mass spectrometry detection (ICP-MS) method were used to measure the Gd concentrations. For all other pharmacokinetic studies, a radioactive isotope, ^{153}Gd-MS-325, was used. Analysis of these samples was performed by measuring the radioactivity in a gamma counter to determine the Gd content of the sample.

- Absorption- Bioavailability-Distribution

All pharmacokinetic studies were based on *iv* administration (the intended route in man) of the compound. The pharmacokinetic profile was examined in the species used for toxicology testing or pharmacology studies (rat, rabbit, dog and monkey). One study was also performed in the baboon.

The data on the pharmacokinetic behaviour of MS-325 after single dose are given in the Table 1 below. The pharmacokinetic profile of MS-325 in the baboon was similar to that seen in the cynomolgus monkey.

Table 1: Comparison of the plasma level-time profiles of MS-325 in selected species

Species	Dose [mmol·kg^{-1}]	Distribution half-life [min]	Elimination half-life [min]	AUC $_{0-\infty}$ [mM x min]	CL [mL/min/kg]	V_{ss} [L/kg]
Rat	0.025	0.87 ± 0.13	22.6 ± 2.87	3.49 ± 0.25	7.20 ± 0.52	0.25 ± 0.04
Rabbit	0.025	3.35 ± 1.12	175 ± 58.2	57.5 ± 19.0	0.47 ± 0.13	0.11 ± 0.01
Cynomolgus * Monkey	0.03	4.22 ± 0.99	172 ± 6.60	59.0 ± 7.50	0.53 ± 0.07	0.12 ± 0.01
Cynomolgus * Monkey	0.03	11.0 ± 3.68	244 ± 5.72	71.4 ± 2.40	0.42 ± 0.01	0.14 ± 0.01
Anaesthetised Monkey	0.025	4.66 ± 3.91	212 ± 148	64.8 ± 41.1	0.56 ± 0.42	0.13 ± 0.03
Human*	0.03	28.8 ± 6.60	1110 ± 180	280 ± 40.8	0.11 ± 0.02	0.15 ± 0.02

AUC $_{0-\infty}$ Area under the curve from time of administration to infinity
CL Total clearance
Vss Volume of distribution at equilibrium
* Both genders combined

The pharmacokinetics of MS-325 was studied after repeated dosing as part of the repeated dose toxicity studies in the rat and cynomolgus monkey, and in the embryo-fetal development studies in the rat and rabbit. In all species, the maximum serum levels at 2-3 minutes after administration were similar on the first and last day (at 4 weeks) of the studies, and increased roughly linearly in relation to the dose. No accumulation was observed.

The organ distribution of MS-325 was studied in the rat, rabbit and cynomolgus monkey. In all species, radioactivity was seen most prominently in the kidney (and bladder), especially at early collecting times. Only in the rat the radioactivity was higher in the intestines, due to the additional hepatobiliary excretion in this species. Residual amounts of radioactivity in the body at 7 or 14 days were extremely low in all species, indicating that almost the entire injected dose was eliminated by then. The organ distribution of MS-325 reflected the elimination routes in the different species, with no relevant retention of Gd observed in any tissue.

- Metabolism

The hepatic metabolism of MS-325 was investigated in human liver microsomes with incubation for 0, 5, 15, 30 and 60 minutes at concentrations of 0.01, 0.1, 1 and 3.6 mM. The highest concentration used was 8 times the maximum concentration of 0.43 mmol·L^{-1} in blood after *iv* administration to normal volunteers of the intended clinical dose. MS-325 was not metabolised by human liver microsomes at any concentration.

Studies in the rat and cynomolgus monkey were performed to investigate the *in vivo* metabolism of MS-325. The animals received a dose of 0.025 mmol·kg^{-1} radiolabelled product. No metabolites were observed in the rat or monkey.

- Excretion

The excretion of MS-325 was studied in the rat, rabbit, dog and cynomolgus monkey. The excretion of MS-325 into the breast milk was studied in lactating rats.

In the rat MS-325 was rapidly eliminated from the body with less than 2% remaining within 24 hours of administration, elimination being complete by 7 days. The primary route of elimination was renal (71%), with 22% excretion through faeces. A low residual activity in the body (<0.5%) at day 7 confirmed that MS-325 did not undergo metabolism, and did not release free Gd. Findings in the rabbit, dog and monkey were similar to those in the rat.

The elimination of MS-325 into the breast milk was investigated following *iv* administration of ^{153}Gd-MS-325 to lactating rat. ^{153}Gd-MS-325 was only transiently found at low levels (<1%) in the breast milk of rats from 1 to 2 hours post-injection. This finding has been reflected in SPC (4.6.).

An in vitro study was performed to determine the dialyzability of MS-325 in the presence of HSA using commercially available dialysis. A single dose of MS-325 (0.43 mmol/L) was added to 4.5 % HSA-containing urea. Urea was cleared from the pre-dialyzer solution in a time dependent manner. The clearance rate was 227 ± 12 mL/min and the time to remove 97 % of the urea was projected to be 1.42 hours at a dialyzer flow of 300 mL/min. MS-325, due to its binding to albumin, was cleared from the predialyzer circuit in a bi-exponential fashion. The clearance rate was 46.8 ± 4.7 mL/min, and the time to achieve a 97 % reduction of the initial MS-325 concentration was projected to be 10 hours based on the raw data or 14.2 hours based on the area under the curve at a dialyzer flow of 300 mL/min. The results indicated that MS-325 could be effectively cleared from the plasma of dialysis patients with a high flux dialyzer filter system.

Toxicology

- Single dose toxicity

Single dose toxicity was tested in mice (*iv* 0.6-6 mmol/kg), rats (5 studies; *iv* 0.03-5 mmol/kg; *iv* 0.1-1.25 mmol/kg fosveset only; intrathecal 0.4-6.4 umol/kg), rabbits (*iv* 2-4 mmol/kg) and monkeys (2 studies; *iv* 0.025-3 mmol/kg). The observed maximum non-lethal dosages were 3 mmol/kg in female mice and $\geq$6 mmol/kg in male mice, 3 mmol/kg in female rats and 2 mmol/kg in male rats, 2 mmol/kg in female rabbits and $\geq$ 3 mmol/kg in monkeys. Observed findings in mice were scabbed areas on the tail, reduced activity, coldness, tremors, and mortality. Findings in rats after *iv* administration were primarily effects in the kidneys (tubular vacuolation, tubular degeneration and nephropathy, and tubular necrosis), and further lethargy and rapid breathing, and mortality. Observed effects in rats after intrathecal administration were ataxia, labored breathing, chromodacryorrhea, convulsions, and mortality. In rabbits, shaking of the heads, mydriasis, an increased respiratory rate, abdominal position, convulsions, nose bleed and mortality were observed. Monkeys showed salivation, retching, vacuolated macrophages in lungs and lymph nodes, and a decreased number of leucocytes. The S-enantiomer (MS-32520-S) caused a slightly higher mortality rate than the R-enantiomer (the actual product) in mice.

- Repeat dose toxicity

No chronic toxicity studies were conducted.

The repeated-dose toxicity studies (with administrations of up to 4 weeks; doses used were 0.01-2.0 mmol/kg in rats, and 0.1-2 mmol/kg in monkeys) revealed vacuolation of renal proximal tubular cells. This finding was observed in the 4-week rat and monkey studies already at doses of 0.03 and 0.5 mmol·kg^{-1}, respectively. The vacuolation of the renal tubular cells at these doses was not associated with an impairment of kidney function; the effect was reversible.

With higher doses (1.0 mmol·kg^{-1} in the repeated dose rat studies and 0.5 mmol·kg^{-1} in the repeated dose monkey studies) formation of vacuoles was also observed in the urinary bladder and urothelium,

as well as in macrophages of lung, lymph nodes, spleen, adrenal medulla, and testes. In rats a dose of 1.5 mmol·kg^{-1} led to kidney tubular necrosis, and increased kidney weights were observed at 1.0 mmol·kg^{-1}. Results of electron microscopy showed that in vacuolated proximal tubular cells of rats, no changes were observed in the organelles, but there was a large storage of material in the cells. These effects were partly reversed after 12 weeks. In monkeys, histologically, only vacuolation was observed in the proximal tubuli and in urothelial tissue, with only minor effects left after 12 weeks recovery.

Mild decrease on haemoglobin concentration, haematocrit and erythrocyte counts was observed in rats (≥ 0.01 mmol·kg^{-1}) and monkeys (≥ 0.5 mmol·kg^{-1}). These effects were reversible. No effects on hemoglobin and red blood cells were observed in single dose studies and no hemolysis was observed in human blood.

Decreases in body weight were observed in rats at a dose of 1.5 mmol·kg^{-1} and in monkeys after a dose of 1.5 mmol·kg^{-1}, resulting in an overall body weight loss for female animals. These findings correlated with frequent emesis in the monkey.

In the liver of high dose monkeys (at about 5 times the expected human exposure), hepatocellular eosinophilic inclusions and periportal hypertrophy and vacuolation were observed after 4 weeks. After 2 weeks, only a weight increase was observed in high dose females. After 1 week and in single dose studies, no liver effects were observed.

The no observed adverse effect level (NOAEL) was based on toxicities other than tubular vacuolation (because the vacuolation of renal proximal tubular cells was considered to be a storage phenomenon due to reabsorption of MS-325 after glomerular filtration). It was determined to be 0.1 mmol·kg^{-1} in the rat and also in the monkey. These NOAELs exceeded the clinical dose at least by a factor of 3 in terms of body weight.

Toxicokinetics

In the rat, rabbit and cynomolgus monkey the AUC was comparable after the first and last (at 4 weeks) dose, and increased linearly with increasing dose. Gadofosveset did not accumulate in rat, monkey nor rabbit after multiple dosing.

- Genotoxicity *in vitro* and *in vivo*

Genotoxicity was investigated in 2 *in vitro* bacterial reverse mutation assays, 2 *in vitro* chromosome aberration assays in Chinese hamster ovary cells and *in vivo* in 2 mouse micronucleus tests in bone marrow. No evidence for a genotoxic potential was found.

- Carcinogenicity (with toxicokinetics)

No carcinogenicity studies were performed.

- Reproductive and developmental studies

Reproduction toxicity studies consisted of a fertility study in rats, several embryotoxicity studies in rats and rabbits preceded by dose-range finding studies, and a peri-/ postnatal study in rats. Intravenous doses ranged between 0.03-2.5 mmol/kg and 0.03-2 mmol/kg in rats (5 studies) and rabbits (4 studies), respectively.

The results of the fertility study did not reveal any impairment of fertility at *iv* doses of up to 1.5 mmol·kg^{-1}. An increased weight of the epididymides and testes was noted for the male rats of the high-dose group. Vacuolated macrophages were visible both in the epididymal and testicular interstitial connective tissue. The number of spermatids per gram testicular tissue was slightly decreased; this was not correlated with a reduction in fertility.

In embryotoxicological study in rats, an increase in the incidence of skeletal variations was found at a maternally toxic dose. In rabbits, an increase in early resorptions was observed starting at a non-

maternally toxic dose. The exposure was 2 times the expected human exposure. There was no effect on the number of foetuses per dam. A few cases (but significantly increased) of hydrocephalus and malrotated limbs were found, all in the high dose (5 times expected human exposure), with only slight maternal toxicity. No effects were observed on pre- and postnatal development in rats.

- Local tolerance

Local tolerance studies were performed in the rabbit covering the intended *iv* route of administration of MS-325, as well as possible paravenous, intra-arterial and intramuscular mis-dosing.

Single intravenous administration was well tolerated in rabbits. After multiple intravenous administration (rabbit studies on embryo-foetal development), dose-dependent local intolerance was observed. Paravenous, intra-arterial, and intramuscular administration of gadofosveset caused mild (paravenous) to moderate (intra-arterial and intramuscular) irritation. Evidence of reversibility was observed.

- Antigenicity

The antigenic and sensitising potential of MS-325 was tested in a systemic anaphylaxis model and a homologous passive cutaneous anaphylaxis model, both in guinea pigs. The studies did not reveal any antigenic potential following iv injection.

- Immunotoxicity

Besides the kidney tubules, macrophages were the most prominent target of MS-325. To investigate whether vacuolation of macrophages caused an impaired function of these cells, function studies were performed in rats assessing the Plaque Forming Cell (PFC) capacity following treatment with sheep red blood cells and the *ex vivo* phagocytosis capacity of the alveolar macrophages. The studies did not reveal any immunotoxic effects.

- Studies on impurities

No specific studies were performed for the toxicological characterisation of the specified impurities free Gd, Gd-EDTA, Gd-HMDTPA, phosphate and lactam.

A study was performed comparing the S-enantiomer of MS-32520, which may occur in small (< 1%) amounts in the final formulation, with R-enantiomer (drug substance). In addition, an acute toxicity study was performed in rats with the ligand excipient (MS-32516-R), the structural equivalent to the drug substance isolated as acid. No significant findings were observed.

- Juvenile toxicity

No studies were performed in juvenile animals.

- Other Studies

To further study the vacuolation of the kidney tubules, a study was performed with a human proximal tubule kidney cell line. Significantly reduced cell viability was found in the highest test concentration of 50 mM. Mitochondrial activity and lysosomal function were reduced at concentrations of ≥ 12.5 mM. The effect on mitochondria was reversible at doses of ≤ 25 mM. Taking into account the clinically relevant doses, MS-325 should show no cytotoxicity towards proximal kidney tubular cells.

An acute toxicity study was performed in rats with a single administration into the lateral brain ventricle to assess the effect of MS-325 in the event of a complete breakdown of the blood-brain barrier. No lethality was observed and there were no clinical signs of toxicity or neurotoxicity.

Ecotoxicity/environmental risk assessment
A phase I risk assessment was made according to the current draft guideline (CPMP/SWO/4447/00) and resulted in a calculated PEC value above the trigger value of 0.01µg/l. Therefore short-term aquatic effect studies (algae, Daphnia and fish) were performed and a refined PEC value based on marketing predictions was calculated. Based on these values, the PEC/PNEC ratio was below 1.

Discussion on the non-clinical aspects

MS-325, a new Gd-based MRI contrast agent, exhibits a long blood residence time as a result of reversible high-degree binding to albumin. In imaging studies, MS-325 enhanced various vascular beds for detection of structural abnormalities such as stenosis. Large vessel visualization was possible at doses of 0.015, 0.025, 0.05 and 0.10 mmol/kg and small vessel visualization at doses of 0.025 mmol/kg and greater.

The safety pharmacology studies showed a 3-10-fold no-effect margin over the recommended human dose of 0.03 mmol/kg. This margin could be estimated to be a safe margin for clinical use. Safety pharmacology studies showed no effects on cardiac activity.

Drug interactions were studied only in respect to protein binding properties and relaxivity changes; since MS-325 was not metabolised, and was therefore not a substrate for metabolising enzymes, enzyme inhibition or induction studies were not performed. These in vitro interactions studies revealed, except for naproxen and ibuprofen, no potential risks for displacement of albumin-bound drugs by MS-325 or vice-versa.

After repeat dosing, no accumulation of MS-325 was observed during daily *iv* administration to the rabbit, rat and monkey during the 4-week administration. MS-325 was cleared from the plasma in a biphasic manner and was mainly distributed within the vascular compartment in the non-rodent species. The similarity between non-human primates and humans, combined with the high degree of human plasma albumin binding, suggested that MS-325 should have a small volume of distribution and a long plasma half-life in man.

MS-325 had a low acute toxicity after *iv* administration. No significant effects on haematology, clinical chemistry and urinalysis parameters, or organ toxicity were observed in the monkey after a single *iv* dose of up to 3.0 mmol·kg^{-1}.

Repeated-dose toxicity studies with MS-325 resulted in vacuolation of the proximal tubular cells of the kidneys, with evidence strongly pointing towards reversibility of the effect. No functional impairment of kidney functions was observed and electron microscopic investigations of the rat kidneys indicated that the observed vacuolation was a storage phenomenon not accompanied by obvious signs of cytotoxicity or necrosis. Similar vacuolation has been observed with iodine and other Gd containing contrast agents. Renal toxicity was higher in rats than in monkeys, because in rats, protein binding is lower and renal clearance is faster, resulting in a higher renal exposure. There was no safety margin for this effect, also not in monkeys. However, in monkeys after administration for 1-4 weeks, at clinical relevant exposures only vacuolation was observed, with creatinine levels increased after 4 weeks administration at 5 times the expected human exposure. No renal effects were observed in single dose studies in monkeys and therefore, it can be regarded unlikely that renal damage would occur in humans after single use at the recommended dose of 0.03 mmol/kg. These findings have been reflected in SPC (5.3.).

A slight decrease in red cell parameters was observed in rats and monkeys. The observed effects were not accompanied by signs of haemolytic anaemia and no haemolytic potential of MS-325 was observed *in vitro*. The findings were shown to be fully reversible and did not occur after a single *iv* administration of 3.0 mmol·kg^{-1}.

The observed reduction of spermatid numbers in the reproduction toxicity studies in the high dose rat group was probably due to a slight weight increase in the testes as a result of vacuolated macrophages

in the interstitial connective tissue. In embryo-fetal toxicity studies an increased incidence of skeletal variations and retardations was observed; this finding has been reflected in SPC (5.3.).

No chronic toxicity studies nor carcinogenicity studies were conducted, since Vasovist is intended to be used for diagnostic investigations which will be carried out only once. No evidence for genotoxic potential was found.

With regard to environmental risk assessment, the PEC/PNEC ratio was below 1, which would normally end the risk assessment process the marketing prediction was not backed up with information. Furthermore, the potential for bioaccumulation of gadolinium was not a concern. However, the CHMP was of the opinion that the applicant should provide a relevant estimate of the environmental concentration measured near hospital outlets; this was requested as a follow-up measure. Based on this data, further information may be needed, as described in 2.3 of this assessment report.

4. Clinical aspects

Introduction

Gadofosveset was developed as an intravenous MRI agent for contrast-enhanced MRA in patients with suspected or known abdominal or limb vascular disease. A total of 18 studies with Gadofosveset were performed. These studies included:

1. Seven clinical phase I/II pharmacological studies
2. Two dose ranging phase II studies performed in patients with vascular disease
3. Four pivotal phase III studies performed in different vascular territories representating different flow states (the aorto-iliac region representing turbulent and high flow, especially near the bifurcation of the distal aorta; the renal arteries, representing flow to an organ; the pedal arteries, where the vessels are smaller in size and the flow is slower than in the other arterial beds).
3. Five additional feasibility studies in other indications included into the safety section but not into the efficacy evaluation (a phase I methodology study in the area of female sexual dysfunction; a feasibility study of imaging of malignant breast lesions; two feasibility studies in coronary MRA; and a feasibility study on myocardial perfusion).

According to the applicant these studies were undertaken according to GCP guidelines and in accordance with the ethical principles as outlined in the Declaration of Helsinki.

Pharmacokinetics

Pharmacokinetic parameters were determined in 167 subjects in six clinical studies. The highest dose level studied (0.15 mmol/kg) was 5-fold higher than the indicated clinical dose of 0.03 mmol/kg. The following pharmacokinetic studies (all single dose studies with intravenous administration of Gadofosveset) were performed:

Healthy volunteers:
- Intravenous bolus infusion
- Intravenous bolus infusion of 30 or 75 seconds

Special populations:
- Subjects with renal impairment (mild, moderate, severe and end stage renal impairment)
- Subjects with moderate hepatic impairment
- Patients with vascular disease without or on chronic warfarin therapy

Separate studies were not undertaken to evaluate effect of age and gender on the pharmacokinetics of Gadofosveset. Effect of age and gender on the pharmacokinetics was evaluated in the pooled results involving 64 of healthy normal volunteers.

- Analytical methods

Inductively coupled plasma atomic emission spectroscopy (ICP-AES) method was developed to measure Gd in human plasma, plasma ultrafiltrate, urine, and faeces. This method was used throughout all pharmacokinetic studies. Methods using HPLC with UV detection were developed to measure intact Gadofosveset in plasma and urine; no method to measure Gadofosveset in feces could be developed. The HPLC-UV method was further modified to allow resolution of the A and B isomers of the drug in urine and plasma. A more sensitive ICP-MS method was developed to measure Gd in urine over extended period and in dialysate.

- Absorption

The absolute bioavailability of Gadofosveset was 100% (*i.v.* administered only). Gadofosveset was developed as an aqueous parenteral solution for rapid intravenous administration, and thus no biopharmaceutic (bioavailability, bioequivalence, food effect, etc.) studies were performed.

- Distribution

The pharmacokinetic parameters are described in the Table 2 below.

Table 2: Pharmacokinetic variables of Gadolinium after single dose intravenous administration of Gadofosveset to healthy volunteers and patients with vascular disease.

Study	Dose (mmol/kg)	Dose[a] (mg/kg)	n	AUC_{inf} (µg.h/ml)	$t_{½}$ (h)	Vss (ml/kg)	CL (ml/h/kg)	CL_R (ml/h/kg)
Healthy volunteers								
MS-325-01A	0.01	1.55	6	274 ± 32	18.0 ± 1.8	147 ± 8	5.7 ± 0.7	4.8 ± 0.3
	0.025	3.93	6	624 ± 95	17.4 ± 2.7	152 ± 14	6.4 ± 1.0	5.1 ± 1.1
	0.05	7.85	6	1040 ± 125	16.6 ± 1.4	174 ± 9	7.7 ± 1.1	5.9 ± 1.3
MS-325-16[b]	0.03	4.65	10	732 ± 107	18.5 ± 3.0	148 ± 16	6.6 ± 1.0	5.5 ± 0.9
	0.05	7.85	10	1077 ± 151	18.8 ± 3.2	162 ± 14	7.4 ± 0.9	6.2 ± 1.0
MS-325-07[b]	0.05	7.86	20	1128 ± 134	18.9 ± 2.7	161 ± 10	7.1 ± 0.8	5.3 ± 0.9
MS-325-01C	0.05	7.86	6	962 ± 132	13.9 ± 1.8	160 ± 9	8.3 ± 1.1	6.1 ± 1.4
	0.075	11.8	6	1227 ± 120	13.4 ± 2.9	179 ± 26	9.7 ± 1.1	7.3 ± 0.9
	0.075c	11.8	6	1327 ± 161	15.0 ± 2.2	184 ± 15	9.0 ± 0.9	7.4 ± 0.7
	0.1	15.7	6	1454 ± 178	14.7 ± 1.8	218 ± 17	10.9 ± 1.2	9.3 ± 1.0
	0.1c	15.7	6	1435 ± 166	14.9 ± 1.0	227 ± 15	11.1 ± 1.4	9.3 ± 1.1
	0.125c	19.7	6	1730 ± 239	14.6 ± 2.9	229 ± 22	11.6 ± 1.6	9.9 ± 1.8
	0.15c	23.6	6	1782 ± 282	13.8 ± 2.4	249 ± 23	13.5 ± 2.2	10.9 ± 1.7
Patients with vascular disease								
MS-325-06[d]	0.05	7.86	10	1166 ± 269	22.2 ± 6.0	172 ± 15	7.0 ± 1.6	5.2 ± 1.3

[a]Dose expressed in mg/kg gadolinium; [b]Only the group with healthy volunteers treated in the study is included in this table; [c]Infusion length of 75 seconds instead of 30 seconds; [d]Only the group with patients with vascular disease without concomitant warfarin therapy is included in this table.

The plasma concentration-time profile of intravenously administered gadofosveset conformed to a two-compartment open model. After *iv* administration of 0.03 mmol/kg dose the mean half-life of the distribution phase ($t_{1/2}\alpha$) was 0.48 ± 0.11 hours and the volume of distribution at steady state was 148 ± 16 ml/kg, roughly equivalent to that of extracellular fluid. At higher doses (0.1-0.15 mmol/kg) Vss was increased because of a decreased fraction of unbound Gadofosveset.

The mean plasma protein binding of Gadofosveset was in the range 80-87% for up to the first 4 hours after injection of 0.03 mmol/kg dose. Directly after rapid *iv* injection, the fraction bound was lower than at later measured lower concentrations, which may have indicated that some time was needed for Gadofosveset to reach equilibrium in protein binding.

- Metabolism and elimination

Gadofosveset was not metabolised. It existed as an equilibrium mixture (1.3 – 1.9:1) of two interconvertible diastereoisomers; both diastereoisomers had comparable activity.

In healthy volunteers, gadofosveset was predominantly eliminated in the urine with 84% (range 79 – 94%) of the injected dose (0.03 mmol/kg) excreted in the urine in 14 days. Ninety-four percent (94%) of the urinary excretion occurred in the first 72 hours. A small portion of gadofosveset dose was recovered in the faeces (4.7%, range 1.1 – 9.3%), indicating a minor role of biliary excretion in the disposition of gadofosveset. After intravenous administration of 0.03 mmol/kg dose renal clearance (5.51 ± 0.85 ml/h/kg) and total clearance (6.57 ± 0.97 ml/h/kg) were similar, and mean terminal elimination half-life was 18.5 ± 3.0 hours. Based on the presented data, no definite conclusions regarding the mechanism of excretion of Gadofosveset could be made.

For in vitro hepatic metabolism data ob human liver microsomes, see Non-clinical section/ Pharmacokinetics/ Metabolism.

- Dose proportionality and time dependencies

The AUC increased less than dose-proportionally within the administered dose range of 0.01-0.15 mmol/kg. The pharmacokinetics of Gadofosveset was influenced by differences in protein binding with different concentrations of Gadofosveset (saturable protein binding). The net free fraction of drug increased with Gadofosveset concentration and at higher dose-levels an increased clearance and volumes of distribution were found.

Inter-individual variability in Gadofosveset pharmacokinetics (AUC and Cl) after intravenous administration was estimated to be 10 – 20 %; intra-individual variability was not evaluated.

Accumulation was not an issue with Gadofosveset as it was administered as single dose only.

- Special populations

An open study with single *iv* dose of 0.05 mmol/kg in 52 subjects showed, that the pharmacokinetics of Gadofosveset was not significantly influenced by mild renal impairment. In moderate and severe renal impairment the half-life increased and plasma clearance decreased with severity of renal impairment. The AUC increased for the moderate and severe impairment group by 2 and 3-fold, respectively.

An open single iv dose study with 0.05 or 0.03 mmol/kg dose in 44 patients with moderate hepatic impairment (Child Pugh B) showed, that plasma pharmacokinetics and protein binding of gadofosveset were not significantly influenced by moderate hepatic impairment A slight decrease in faecal elimination of gadofosveset was seen for the hepatic impaired subjects (2.7%) compared to normal subjects (4.8%). In one subject with moderate hepatic impairment and abnormally low serum albumin, total clearance and half-life of gadofosveset was indicative of faster clearance compared to

subjects with moderate hepatic impairment and normal serum albumin levels. No pharmacokinetic data was available in subjects with severe hepatic impairment.

Gender and age had no significant influence on the pharmacokinetics of Gadofosveset based on an analysis of pooled data from 64 healthy volunteers. The influence of weight and race on the pharmacokinetics of Gadofosveset were not studied. No data were obtained in adolescents or children.

- Pharmacokinetic interaction studies

In vitro
Effect of Gadofosveset on protein binding of S-ketoprophen, piroxicam, diazepam, ibuprofen, R-ketoprophen, naproxen, diclofenac, digitoxin, propranolol, verapamil and warfarin was investigated in two in-vitro studies. Only increase of unbound warfarin in the presence of Gadofosveset was found. A second study was carried out with Gadofosveset and warfarin only. In this study no influence on protein binding of warfarin was found in the presence of Gadofosveset. In a human liver microsome study no metabolism of Gadofosveset was found.

An in vitro inhibition study with gadofosveset and the ligand fosveset on the metabolism of established substrates of the CYP450 enzyme system using human liver microsomes was carried out in order to investigate whether MS-325 can affect metabolism of compounds which are metabolised by the P450 enzyme system (non-competitive inhibition); this study was requested by the CHMP during the evaluation of the dossier. The positive controls confirmed the expected metabolic activity of the used preparations of human liver microsomes. No inhibitory effects of gadofosveset on CYP1A2 (Phenacetin), CYP2A6 (Coumarin), CYP2C8 (Paclitaxel), CYP2C9 (Diclofenac), CYP2C19 (S-mephenytoin), CYP2D6 (Bufuralol), CYP2E1 (Chlorzoxazone) and CYP3A4 (Testosterone) were observed, resulting in no measureable IC50 within the tested concentration range (0-300 µM) for these 8 isoenzymes. No inhibitory effects of the ligand fosveset on CYP1A2, CYP2A6, CYP 2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4 were observed at a concentration of 1 µM.

In vivo
Effect of Gadofosveset on the pharmacokinetics of warfarin was studied in one study. In a group of twelve patients on warfarin therapy, unbound fractions of *R*- and *S*-warfarin in the pre-dose through samples were not affected after administration of 0.05 mmol/kg dose of Gadofosveset. Further, the concomitant use of warfarin did not affect pharmacokinetics of Gadofosveset.

Pharmacodynamics

Clinical pharmacodynamics was studied in four clinical studies.

- Mechanism of action

Gd is a rare earth metal that causes signal enhancement on MRI images by interacting with water molecules and shortening the T1 of the water molecules that interact with the Gd atom. The binding of a MR contrast agent to macromolecules (e.g. serum albumin in the case of Gadofosveset) leads to a high relaxivity and contrast. Following intravenous administration of a 0.03 mmol/kg dose (the proposed clinical dose), 80 to 90% of MS-325 was reversibly bound to albumin. As a result, MS-325 remained in the circulation for a long period and thus prolonged enhancement of MRI. Due to high albumin binding the contrast agent began to spin slowly, at the rate albumin spins, increasing relaxivity and thus with brighter signal than would be possible with freely circulating Gd.

- Primary and Secondary pharmacology

In MS-325-01A-study Gadofosveset was administered as an iv bolus at 0.01, 0.025, or 0.05 mmol/kg in 27 healthy adult volunteers. Gadofosveset caused a significant increase in the plasma Δ1/T1 at all dose levels and at all time points. The observed relaxation rate kinetics was consistent with the

kinetics of plasma concentration profiles. As the dose was increased from 0.01 to 0.025 mmol/kg, there was a 2.3 times mean increase in relaxation rate. However, for dose increase from 0.025 to 0.05 mmol/kg, the mean increase in the relaxation rate was 1.5 times. This resulted in a significantly lower dose-corrected Δ1/T1 AUC for the 0.05 mmol/kg dose, as compared to lower doses. Decreased plasma protein binding at higher plasma concentrations at higher dose was the likely explanation for the relatively reduced pharmacodynamic effectiveness of Gadofosveset at 0.05 mmol/kg dose compared to the two lower dose levels tested in the study.

In MS-325-01B-study Gadofosveset was administered as an iv bolus at 0.05 mmol/kg in 17 healthy adult volunteers. Early arterial phase imaging demonstrated significantly improved enhancement of three vascular regions; abdomen, carotids and peripheral. All post-dose images were qualitatively graded as diagnostic, and an increase in image quality was always noted from pre- to post-Gadofosveset images. Steady-state images were obtained up to 60 minutes post injection. Since the images could be obtained for a longer time due to slower elimination of Gadofosveset, the need for repeat dose on short term would be unlikely.
Quantitatively, the mean early (approximately 5 minute) arterial signal-to-noise ratio and contrast-to-noise ratio showed modest but statistically significant decrease of between 11-15% on the delayed (60 minute) images.

In MS-325-06-study Gadofosveset was evaluated in the setting of arterial vascular occlusive disease in patients on warfarin therapy. 12 of the patients were on warfarin therapy and 10 were not on warfarin therapy. Change in plasma relaxation rate was not significantly different between the two groups. Additionally, the anticoagulant activity of warfarin was not affected by Gadofosveset administration.

In MS-325-18-study in end-stage dialysis patients it was shown that after bolus intravenous administration of 0.05 mmol/kg dose in patients requiring three times a week haemodialysis using high-flux filter, at the end of third dialysis session, the plasma concentration had declined to less than 15 % of the Cmax. During the dialysis sessions the mean half-life of plasma concentration decline was in the range 5-6 hours. The mean dialysis clearance was between the range of 16 – 32 ml/h/kg. The high-flux dialysis filter was more efficient compared to the low-flux filter.

Also an in vitro study was performed to determine the dialyzability of Gadofosveset in the presence of 4.5 % HSA using commercially available dialysis equipment. A single dose of Gadofosveset was added to 4.5 % HSA-containing urea. Urea was cleared from the pre-dialyzer solution in a time dependent manner. The clearance rate of Gadofosveset was 46.8 ± 4.7 mL/min, and the time to achieve a 97 % reduction of the initial Gadofosveset concentration was projected to be 10 hours based on the raw data or 14.2 hours based on the area under the curve at a dialyzer flow of 300 mL/min. Thus, gadofosveset could be effectively cleared from plasma of dialysis patients with a high flux dialyzer filter system.

For in vitro studies on binding of Gadofosveset to HSA, see Nonclinical section/ Primary pharmacodynamics/ In vitro.

Clinical efficacy
Clinical program to demonstrate efficacy included two dose ranging phase II studies and four main phase III studies performed in different vascular territories representing the described flow states (see the Table 3 below).

Table 3: The Phase II/III efficacy studies

Study Number	Study Title	MS-325 Dose (mmol/kg)	Number of Patients (ITT population)
MS-325-02	A Phase II Study of the Safety and Preliminary Efficacy of MS-325 Enhanced Magnetic	0.01 0.03	14 28

	Resonance Angiography in Carotid and Peripheral Arteries	0.05	31
MS-325-09	A Phase II, Randomized, Multicenter, Comparative, Dose-ranging, Placebo-controlled Study to Determine the Safety and Efficacy of MS-325-enhanced MRA for Evaluation of Aortoiliac Occlusive Disease in Patients with Known or Suspected Peripheral Vascular Disease	0 (placebo) 0.005 0.01 0.03 0.05 0.07	37 44 34 39 40 39
MS-325-12	A Multicenter, Comparative, Phase 3 Study to Determine the Safety and Efficacy of MS-325-Enhanced MRA for Evaluation of Aortoiliac Occlusive Disease in Patients with Known or Suspected Peripheral Vascular Disease	0.03	268
MS-325-13	A Multicenter, Phase III Study to Determine the Safety and Efficacy of MS-325-enhanced MRA in Patients with Suspected Peripheral Vascular Disease	0.03	175
MS-325-14	A Phase III Multicenter Study to Determine the Safety and Efficacy of MS-325-enhanced MRA in Patients with Known or Suspected Renal Arterial Disease	0.03	136
MS-325-15	A Multicenter, Randomized, Open-label, Two-dose, Comparative, Phase III Study to Determine the Safety and Efficacy of MS-325-enhanced MRA in Patients with Known or Suspected Pedal Arterial Disease	0.03 0.05	93 87

- Dose response studies

The objectives, treatment, design and endpoint assessment of the dose ranging phase II studies were similar to the four main phase III studies. The primary efficacy analysis was based on improved sensitivity, specificity, and accuracy of Gadofosveset enhanced MRA when compared with unenhanced MRA taking XRA as standard of reference (SOR) for the detection of clinically significant stenosis (≥ 50%). Secondary efficacy parameters included a direct comparison of the percent stenosis measurement by MRA and by XRA; an receiver operating characteristic (ROC) analysis based on a grading of stenosis on a qualitative scale of 1-5 for the presence of disease; diagnostic confidence; the number of uninterpretable MRA exams, and a separate interpretation of all the MRA and XRA data by interventionalists (vascular surgeons) to determine how the images would be used in patient management decisions.

The MS-325-02-study examined the safety and preliminary efficacy for detection of vascular stenosis in three dose groups (0.01, 0.03 and 0.05 mmol/kg) in one of three regions: carotid artery, iliac (including the common femoral artery), and superficial femoral arteries. Safety analysis was done for all 81 patients enrolled, whereas efficacy data were analysed for 73 patients. The study showed a dose-related post-contrast MRA enhancement (signal to noise ratio and contrast to noise ratio), but were inconclusive with regard to the determination of a minimum effective dose of Gadofosveset taking into account the diagnostic test based characteristics (sensitivity, specificity, and accuracy). Therefore a second phase II dose response study (MS-325-09) was designed.

The randomized, double-blind study MS-325-09 included 238 patients who received one of five doses of Gadofosveset or placebo. This study evaluated the safety, dose response, and the minimal effective

dose of Gadofosveset for detection of vascular disease (defined as clinically significant stenosis, i.e., ≥ 50%) in four arteries of the aortoiliac region on both the left and right sides: infra-renal abdominal aorta, common iliac artery, external iliac artery, and the common femoral artery. All three blinded readers demonstrated a significant dose response ($p < 0.001$) for improvements of post-contrast MRA compared with pre-contrast. The peak increase in AUC (i.e. an increase in overall diagnostic improvement compared to pre-contrast MRA) was centered at a dose of 0.03 mmol/kg.

The percentage diseased vessels (> 50% stenosis) was 15% (240 diseased vessels out of 1585 vessels in 233 patients) equally distributed over the different MS-325 dose groups. Using ≥ 50% stenosis as the definition of clinically significant disease, improvements in specificity and sensitivity for post-contrast MRA compared to pre-contrast MRA generally followed the same dose shape as that produced by the ROC analysis. Specificity improved for all three readers up to and including the 0.03 mg/kg dose. Due to the smaller number of diseased vessels compared to nondiseased vessels led to a greater variability among sensitivity estimates.

Ninety patients across the five doses (plus placebo) had at least one renal artery that was interpretable on the XRA and had MR vessels within the field of view. The renal artery data followed the same dose trends as the aortoiliac arteries. Both specificity and overall accuracy for both sides independently showed a statistically significant dose response ($p < 0.001$). There was an insufficient number of diseased renal arteries in this population to assess sensitivity independently.

However, although the signal ratio for the ascending lateral circumflex of the femoral artery compared with the thigh muscle showed a statistically significant dose response it could not be ruled out that the 0.05 mmol/kg dose in these small vessels would provide higher clinical efficacy in small, slow-flow vessels compared with the 0.03 mmol/kg dose. Additional clinical data for the 0.03 mmol/kg and 0.05 mmol/kg dose groups in the pedal arteries of the feet, representing the small, slow flow arteries, were therefore collected in the pivotal study MS-325-15. In that study, the dose selection was reinforced for low-flow arteries by the direct comparison of sensitivity, specificity and accuracy of the 0.03 mmol/kg and 0.05 mmol/kg dose groups. In this study, the 0.03 mmol/kg dose performed numerically not worse than the 0.05 mmol/kg dose.

- Main studies

Methods

The trials were controlled and followed the principals of US FDA draft guidance as well as European guidance (CPMP/EWP/1119/98) regarding blinded reading for establishing the effectiveness of medical imaging agents.

In all Phase III trials, the clinical methodology was the same:

- each trial was designed to evaluate and quantify the improvement provided by the administration of MS-325 compared with MRI without contrast enhancement. Catheter angiography (XRA) was used as the standard of reference (SOR),
- all images were acquired using prospectively defined MRI protocols,
- the primary analysis of the trials used independent, blinded readers, each of whom interpreted all images of a given modality in a given study,
- blinded readers were board-certified in their respective field of expertise, had no prior affiliation with the sponsor, and had not participated in other MS-325 trials,
- each trial had a completely different set of blinded readers; a total of 32 independent blinded readers (24 radiologists, 8 vascular surgeons) were used,
- the independent MRA and XRA blinded assessments followed a prospectively designed and approved blinded assessment methodology,
- images were randomized and no other clinical information was provided to the blinded readers.
- for MRA blinded readers, a randomization scheme was used that put at least 1/4 of the image cases between the pre- and post-contrast images of the same patient in order to minimize recall bias,

- the SOR was based on the independent interpretations of at least two XRA blinded readers; no consensus reading was performed in determining the SOR.

The Phase II and III trials were described as controlled studies on the basis of having:
- an active comparison (pre-contrast MRA performed with the device alone),
- a clinical standard of reference (SOR) using XRA for determining the presence of clinically significant stenosis (≥ 50%) in patients with known or suspected vascular disease, and,
- the use of blinded, independent readers to read all MRA and XRA images.

No other Gd based MRI contrast agents were included in the Phase II and III trials as "active comparators".

Study participants

Patients enrolled in the studies had either known or suspected vascular disease (aortoiliac, renal, or pedal), and they were patients for whom an XRA examination was planned. The MRA and XRA studies were performed within three to 30 days of each other, and no interventions were done in the period between the studies. Patients were 18 or more years of age. Patients were excluded from the study if they had had a major cardiovascular event within 30 days prior to study randomization, a history of renal transplant or hemodialysis, a history of hemoglobinopathy or specific magnetic resonance exclusion criteria, such as the presence of a pacemaker, internal defibrillator or ferromagnetic intra-cranial aneurysm clip. Patients were also excluded if they had a hypersensitivity to gadolinium based contrast agents or had previously received Gadofosveset. Patients could not have received iodine or other contrast agents within three days prior to or following Gadofosveset administration. Finally, patients who had a surgical intervention within 30 days prior to drug administration were excluded from the studies.

Treatments
See table "Phase II/III efficacy studies" above.

The selection of the 0.03 mmol/kg dose og Gadofosveset was based on the analysis of the dose-ranging studies MS-325-02 and MS-325-09. For all vessel beds studied, representative of turbulent flow, slow flow and flow to an organ, a 0.03 mmol/kg dose of Gadofosveset was used in the setting of the minimum effective dose for MRA. For the SOR the method which represents the current standard of care in vascular diagnostics for the whole spectrum of vascular territories was intra-arterial XRA using catheter technique chosen. No other reference procedure, though proved efficacious in some vessel regions, could have been applied to all regions. Thus, only XRA was considered the appropriate SOR. In compliance with regulatory guidance, comparison to unenhanced MRA (device alone) was included in the pivotal study program.

Objectives
The study objectives in all the main studies were to investigate delineation of normal vascular anatomy and vascular pathology such as stenotic or aneurysmatic vessels, and to assess the degree of clinically important abnormalities.

Outcomes/endpoints

The primary efficacy analysis for all studies was based on the sensitivity, specificity, and accuracy for the detection of clinically significant stenosis (≥ 50%) on XRA in all target vessels from patients in the 0.03 mmol/kg dose group. The 50% cut-off for the definition of significant disease followed well-established clinical guidelines and literature.

Additional secondary endpoints were used to provide more data to compare post-contrast MRA to the clinical standard, catheter angiography, for the anatomic characterization of these lesions, including: (a) a direct comparison of the percent stenosis measurement by MRA and by XRA; (b) an ROC

analysis based on a grading of stenosis on a qualitative scale of 1-5 for the presence of disease; and (c) a separate interpretation of all the MRA and XRA data by interventionalists (vascular surgeons) to determine how the images would be used in patient management decisions. In the patient management blinded read, the vascular surgeons were provided with both the images and the results from blinded radiologists in order to mimic clinical practice.

Sample size

The primary statistical analysis was based on the difference in sensitivity and specificity, using XRA as SOR, between post-contrast MRA and pre-contrast MRA. A modified McNemar test was used, which accounts for clustering. Sample size was based on the assumptions that around 80% of the vessels would not have clinically significant stenoses according to a previous study, expected sensitivities (specificities) for pre-contrast and post-contrast MRA would be 70% and 85% respectively, the correlation would be 0.15 for pre-contrast and post-contrast, and the intraclass correlation coefficient would be 0.70 for the vessels within each patient and for a power of 90% for sensitivity, specificity, and accuracy.

Statistical methods

The analysis of all Phase III studies followed an intent-to-treat (ITT) methodology. The ITT-population was defined as all patients who were enrolled in the study, received study drug and underwent a XRA and post-administration MRA. Vessel data was excluded from any analysis if the XRA data were missing or were uninterpretable for that vessel. Uninterpretable or missing MRA data were considered inaccurate compared to the XRA when the corresponding XRA images were interpretable. In the quantitative comparison of difference in percent stenosis measurement, the percent stenosis for vessels uninterpretable by MRA was imputed to a value that maximized the difference between the MRA and XRA values. Similarly, in the vascular surgeon blinded read, patients whose MRA data were considered inadequate for treatment planning were considered mismatches to the XRA data. The primary efficacy analysis was based on the blinded reader assessments for sensitivity, specificity, and accuracy for the detection of clinically significant stenosis (~ 50%) in all target vessels for the post-contrast MRA procedure compared with pre-contrast MRA using the adjudicated XRA as the SOR. The test for significance was based on McNemar's test modified for the potential effects of correlations among vessels within a given patient. A patient-based analysis of the primary efficacy variables was performed as a supportive analysis to the vessel-based analysis described above.

The endpoints calculated were:
Sensitivity – The percentage of times that the MRA result was positive given that the XRA was positive. The denominator for sensitivity is the number of vessels with positive results from the XRA.
Specificity – The percentage of times that the MRA result was negative given that the XRA was negative. The denominator for specificity is the number of vessels with negative results from the XRA.
Accuracy – The percentage of times that the MRA result matches the XRA result. For accuracy the denominator is all vessels assessed by the XRA readers excluding those that were uninterpretable, i.e. the sum of the denominators for sensitivity and specificity.

The secondary efficacy variables were the following:
-ROC curves based on the blinded reader's qualitative impression of the presence of clinically significant disease;
-Blinded reader confidence of diagnosis of disease
-Percentage of uninterpretable vessels
-Absolute differences in percent stenosis between blinded MRA reads and average blinded XRA reads as well as between individual blinded XRA readers
-Agreement of location of stenosis between MRA and XRA blinded reads for all clinically significant vessels/segments
-Inter-reader agreement
-Agreement between the two blinded XRA readers

-Sensitivity, specificity, and accuracy for the detection of aneurysm
-Vascular surgeon agreement on patient management decisions
-Institutional MRA readers' sensitivity, specificity, and accuracy in the detection of vascular disease in all vessels from patients receiving the Gadofosveset 0.03 mmol/kg dose using the institutional XRA reads as standard of reference

Study designs

MS-325-12-study was an open-label study including 274 patients with known or suspected vascular disease of the aortoiliac region who received a single dose of 0.03 mmol/kg of Gadofosveset. This study evaluated the safety and efficacy of Gadofosveset for the detection of vascular disease in four arteries of the aortoiliac region on both the left and right sides: infra-renal abdominal aorta (IRAA), common iliac artery (CIA), external iliac artery (EIA), and common femoral artery (CFA). Data from this study were considered representative of vessels that have turbulent flow.

MS-325-13-study was an open-label study including 178 patients with known or suspected vascular disease of the aortoiliac region who received a single dose of 0.03 mmol/kg of Gadofosveset. This study evaluated the safety and efficacy of Gadofosveset for the detection of vascular disease in four arteries of the aortoiliac region on both the left and right sides: IRAA, CIA, EIA, and CFA. In this study, analysis of the location of stenosis within a particular vessel segment was added as a secondary efficacy endpoint. Data from this study was considered representative of vessels that have turbulent flow. Taken together with MS-325-12, this study was designed to determine the repeatability of results for peripheral vascular disease detection by post-contrast MRA.

MS-325-14-study was an open-label study with the same study design as studies MS-325-12 and MS-325-13. This study evaluated the safety and efficacy of Gadofosveset in 145 patients for the detection of vascular disease in the renal artery (proximal and distal segments) and accessory renal arteries (where present).

MS-325-15-study was an open-label study including 185 patients with pedal arterial disease. This study evaluated the safety and efficacy of Gadofosveset for the detection of vascular disease in four arteries on either the left or right side: posterior tibial (below the ankle), dorsalis pedis, and the medial and lateral plantar arteries. It was designed as a randomized, open-label two-dose study using Gadofosveset doses of 0.03 mmol/kg (93 patients) and 0.05 mmol/kg (83 patients). The additional dose was included because the previous MS-325-09-study in the ascending lateral circumflex of the femoral artery had suggested nearly equivalent performance between the 0.03 mmol/kg and 0.05 mmol/kg doses and because it had been suggested that the higher dose might be required to show efficacy in the slow flow inherent to the pedal vascular bed.

Results

Participant flow

Patient disposition for the Phase III-studies (0.03 mmol/kg dose) is given in the Table 4 below. Overall, 94% of ITT patients were considered evaluable for the blinded reader efficacy analysis (i.e., had XRA images that were interpretable and thus contributed to the SOR), and 99% of ITT patients were considered evaluable for the institutional reader analysis.

Table 4: Summary of patient disposition for the 0.03 mmol/kg dose group in phase III

	Phase III Studies											
	MS-325-12		MS-325-13		MS-325-14		MS-325-15 [1]			Combined [2]		
Variable	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**	**N**	**(%)**		**n**	**(%)**	
Dosed	274		178		145		96			693		
ITT Population	268	(100.0)	175	(100.0)	136	(100.0)	93	(100.0)		672	(100.0)	
Blinded Reader Evaluable*	251	(93.7)	173	(98.9)	127	(93.4)	80	(86.0)		631	(93.9)	
Institutional Reader Evaluable#	268	(100.0)	175	(100.0)	132	(97.1)	90	(96.8)		665	(99.0)	

*ITT patients who had at least 1 interpretable image according to X-ray result.
ITT patients who had at least 1 interpretable image according to the Institutional Reader X-ray result.

Outcomes and estimation

The primary efficacy analysis was based on the sensitivity, specificity, and accuracy for the detection of clinically significant stenosis (≥ 50%) in all target vessels from patients in the 0.03 mmol/kg dose group in the Phase III studies. Results for sensitivity and specificity, and for accuracy are summarized by study and reader in the two below Tables 5-6. Gadofosveset improved diagnostic performance in terms of accuracy (range: 8.1-28.7%), sensitivity (6.3-41.5%) and specificity (8.4-29.3%) when compared with unenhanced MRA for assessment for detection of stenosis greater than 50% in the lower extremity for arterial disease (iliac and renal). In the pedal study the increase in accuracy varied between 7.0 and 17.7% and for specificity between 8 – 35%; there was nonconsistent improvement of sensitivity.

Table 5: Phase III blinded read sensitivity and specificity for post-contrast MRA in the 0.03 mmol/kg dose group by study and reader (intent-to-treat population)

Study (Disease Type) MRA Reader Variable	**Number of Patients [1]**	**Number of Vessels [1]**	**Post-contrast (%)**	**Pre-contrast (%)**	**Difference (%) [2]**	**p-value [3]**
MS-325-12 (AIOD)						
MRA Reader A						
Sensitivity	140	237	80.2	62.0	18.1	<0.001
Specificity	250	1409	84.5	75.1	9.4	<0.001
MRA Reader B						
Sensitivity	140	237	73.0	66.7	6.3	0.060
Specificity	250	1409	93.2	84.8	8.4	<0.001
MRA Reader C						
Sensitivity	140	237	60.8	41.8	19.0	<0.001
Specificity	250	1409	95.3	75.4	19.9	<0.001
MS-325-13 (AIOD)						
MRA Reader A						
Sensitivity	85	146	82.9	52.1	30.8	<0.001
Specificity	172	1018	80.0	70.7	9.2	0.001
MRA Reader B						
Sensitivity	85	146	84.2	60.3	24.0	<0.001

Study (Disease Type) MRA Reader Variable	Number of Patients [1]	Number of Vessels [1]	Post-contrast (%)	Pre-contrast (%)	Difference (%) [2]	p-value [3]
Specificity	172	1018	83.0	74.5	8.5	<0.001
MRA Reader C						
Sensitivity	85	146	70.5	48.6	21.9	<0.001
Specificity	172	1018	90.1	78.2	11.9	<0.001
MS-325-14 (Renal)						
MRA Reader A						
Sensitivity	40	53	56.6	30.2	26.4	0.005
Specificity	116	229	77.3	48.0	29.3	<0.001
MRA Reader B						
Sensitivity	40	53	66.0	41.5	24.5	0.009
Specificity	116	229	81.7	59.0	22.7	<0.001
MRA Reader C						
Sensitivity	40	53	64.2	22.6	41.5	<0.001
Specificity	116	229	82.5	57.2	25.3	<0.001
MS-325-15 (Pedal)						
MRA Reader A						
Sensitivity	72	200	93.0	77.0	16.0	<0.001
Specificity	53	116	59.5	38.8	20.7	0.010
MRA Reader B						
Sensitivity	72	200	77.5	86.5	-9.0	0.062
Specificity	53	116	66.4	31.9	34.5	<0.001
MRA Reader C						
Sensitivity	72	200	78.5	78.0	0.5	0.919
Specificity	53	116	62.9	28.4	34.5	<0.001

[1] Patients with at least one abnormal vessel by X-ray and vessels considered abnormal by X-ray are included in the sensitivity calculation. Patients with at least one normal vessel by X-ray and vessels considered normal by X-ray are included in the specificity calculation. Uninterpretable MRA values were considered inaccurate for this analysis.
[2] Difference=(Post-contrast MRA - Pre-contrast MRA).
[3] Modified McNemar test

Table 6: Phase III blinded read accuracy for post-contrast MRA in the 0.03 mmol/kg dose group by study and reader (intent-to-treat population)

Study (Disease Type) MRA Reader	Number of Patients [1]	Number of Vessels [1]	Post-contrast (%)	Pre-contrast (%)	Difference (%) [2]	p-value [3]
MS-325-12 (AIOD)						
MRA Reader A	251	1646	83.8	73.2	10.6	<0.001
MRA Reader B	251	1646	90.3	82.2	8.1	<0.001
MRA Reader C	251	1646	90.3	70.6	19.7	<0.001
MS-325-13 (AIOD)						
MRA Reader A	173	1164	80.3	68.4	11.9	<0.001
MRA Reader B	173	1164	83.2	72.7	10.5	<0.001
MRA Reader C	173	1164	87.6	74.5	13.1	<0.001
MS-325-14 (Renal)						
MRA Reader A	127	282	73.4	44.7	28.7	<0.001
MRA Reader B	127	282	78.7	55.7	23.0	<0.001

MRA Reader C	127	282	79.1	50.7	28.4	<0.001
MS-325-15 (Pedal)						
MRA Reader A	80	316	80.7	63.0	17.7	<0.001
MRA Reader B	80	316	73.4	66.5	7.0	0.126
MRA Reader C	80	316	72.8	59.8	13.0	0.004

[1] Accuracy population was the total number of interpretable vessels evaluated by XRA. Uninterpretable MRA values were considered inaccurate for this analysis.
[2] Difference=(Post-contrast MRA - Pre-contrast MRA).
[3] Modified McNemar test

Ancillary analyses

For the patient-weighted analysis, 10 of 12 blinded readers demonstrated significant improvements in sensitivity (range of improvements: 8% to 40%; $p < 0.04$ for improvement compared with pre-contrast), 11 of 12 readers showed significant improvements in specificity (range of improvements: 7% to 32%; $p < 0.02$ for improvement compared with pre-contrast for 11 of the 12 readers), and 11 of 12 readers showed significant improvements in accuracy (range of improvements: 8% to 29%; $p < 0.01$ for improvement compared with pre-contrast). A summary of the supportive analysis using patient-weighted estimators, based on an analysis of all readers combined for each phase III study, is presented in the Table 7 below. When the results from the three blinded MRA readers for each study were combined, significant improvements in sensitivity were seen in studies MS-325-12, MS-325-13, and MS-325-14; significant improvements in specificity were seen in all four phase III studies; and significant improvements in accuracy were seen in all phase III studies.

Table 7: Blinded Read Results of MS-325 Sensitivity, Specificity, and Accuracy for the 0.03 mmol/kg Dose Group of Phase III Studies - Patient-weighted Analysis for All Readers Combined (Intent-to-Treat Population)

Study (Disease Type) Variable [1]	Number of Patients	Post-contrast (%)	Pre-contrast (%)	Difference (%) [2]	p-value [3]
MS-325-12 (AIOD)					
Sensitivity	140	71.1	56.6	14.5	<0.001
Specificity	250	90.3	78.7	11.7	<0.001
Accuracy	251	88.1	75.3	12.8	<0.001
MS-325-13 (AIOD)					
Sensitivity	85	78.5	53.3	25.2	<0.001
Specificity	172	83.0	73.9	9.1	<0.001
Accuracy	173	83.6	71.9	11.8	<0.001
MS-325-14 (Renal)					
Sensitivity	40	62.1	34.2	27.9	<0.001
Specificity	116	80.2	55.2	25.0	<0.001
Accuracy	127	78.2	51.1	27.1	<0.001
MS-325-15 (Pedal)					
Sensitivity	72	80.6	79.3	1.3	0.722
Specificity	53	56.6	31.1	25.4	<0.001
Accuracy	80	75.3	62.9	12.4	<0.001

Note: Uninterpretable MRA values were considered inaccurate for this analysis. Patient-weighted estimators are used for the summary analysis while vessel-weighted ratio estimators are used for the primary analysis Sensitivity, specificity, and accuracy are patient-weighted estimates.
[1] Sensitivity, specificity, and accuracy for a patient's data are averages of the individual readers for

the same patient.
[2] Difference=(Post-contrast MRA - Pre-contrast MRA).
[3] P-value is from the paired t-test.

Impression of presence of clinically significant disease
For each study, the ROC data were consistent with the sensitivity and specificity data from the primary stenosis measurement and showed that the readers' qualitative assessment of the presence/absence of clinically significant disease improved after MS-325 administration.

Confidence of diagnosis of disease
All readers in all studies showed improved confidence using post-contrast MRA compared with pre-contrast MRA. For all readers in the phase III studies except one reader in MS-325-15, the improvement was statistically significant.

Uninterpretable images
For all MRA readers in all studies, the proportion of uninterpretable vessels decreased substantially after the administration of MS-325. For pre-contrast MRA, the average proportion of uninterpretable vessels across MRA readers ranged from 10% to 34%; for post-contrast MRA, the average proportion varied from 1% to 3%. The post-contrast level of uninterpretability was better than that observed for the XRA readers. Specifically, the average proportion of uninterpretable vessels across XRA readers was between 3% and 8% for all studies.

Percent stenosis and differences in percent stenosis
The absolute agreement between MRA and XRA improved substantially for all MRA blinded readers in each study after Gadofosveset administration, based on the decrease in the absolute difference in percent stenosis for pre-contrast and post-contrast MRA. For MS-325-12, pre-contrast values ranged from 18% to 29%, post-contrast values ranged from 11% to 16%, and the difference between XRA readers was 14%; for MS-325-13, the ranges were 28-31% for pre-contrast, 16-18% for post-contrast, and 16% between XRA readers; for MS-325-14, the ranges were 31-41% for pre-contrast, 14-15% for post-contrast, and 11% between XRA readers; and for MS-325-15, the ranges were 34-40% for pre-contrast, 21-27% for post-contrast, and 19% between XRA readers.

Location of stenosis
For all MRA readers in studies MS-325-13 and MS-325-14, the location match increased substantially from pre-contrast MRA (ranges: 60-73% for MS-325-13; 22-56% for MS-325-14) to post-contrast MRA (ranges: 88-95% for MS-325-13; 62-85% for MS-325-14). In MS-325-15, location match was similar for post-contrast MRA (range 60-71%) and pre-contrast (range 51-72%).

Inter-reader agreement
The generalized kappa statistic for the inter-reader agreement analysis indicated a fair level (0.3297-0.4236) of inter-reader agreement among the blinded MRA readers.

Presence of aneurysm
In the AIOD study MS-325-13, the only study in which there was a significant number of X-ray proven aneurysms, the sensitivity for detecting aneurysms improved substantially, from 53-73% for pre-contrast MRA to 93-100% for post-contrast MRA. This improvement was statistically significant for two of the three readers in that study. Across all studies all nine readers showed improvement in specificity (range: 1-33%) from pre-contrast to post-contrast MRA, and the improvement was statistically significant ($p \leq 0.002$) for eight of the nine readers. Similarly, all nine blinded MRA readers across the three studies improved in the accuracy for the detection of aneurysm from pre-contrast MRA to post-contrast MRA (range of improvements: 1-33%), and the improvement was statistically significant for eight of the nine readers ($p \leq 0.002$).

Next course agreement of patient Management

For all readers in all studies, the vascular surgeon readers agreed with XRA more often when using post-contrast MRA than when using pre-contrast MRA. For six of the eight readers, the improvement was substantial (> 15%) and statistically significant ($p < 0.001$).

Institutional reader analyses
Across all four phase III studies, the institutional reader results were consistent with the blinded reader results.

Impact on therapeutic decisions
The applicant was requested by the CHMP to provide a synthetic vision of the impact of MRA with Gadofosveset on therapeutic decisions and the consequences of false positive and false negative results.

In aortoiliac disease studies the false positive rates for MS-325 enhanced MRA ranged from 5-20% as compared to 15-29% for unenhanced MRA. The false negative rates ranged from 16-39% for MS-325 enhanced MRA and 33-58% for unenhanced MRA. For renal arteries the false positive rates were 18-23% for enhanced MRA and 41-52% for unenhanced MRA; the false negative rates were 34-43% for enhanced MRA as compared to 59-77% for unenhanced MRA. Similar results were observed for the pedal trial where false positive rates for enhanced MRA were 34-41% as compared to 61-72% for unenhanced MRA.

Also two vascular surgeons reviewed the MRA and XRA images independently to decide the next course of management in each of the four main trials. The vascular surgeons blinded read data was re-analysed to generate false positive and false negative results for enhanced MRA. False positive results were defined as when the patient was incorrectly considered for treatment and false negative results as when the patient was incorrectly not considered for further treatment taking XRA treatment decisions as SOR. 75-79% of the patients would have undergone unnecessary diagnostic procedure based on unenhanced MRA compared to 21-58% for MS-325 enhanced MRA.

Impact of different cut-off values with regard to clinically significant stenosis
The definition of clinically significant stenosis (at least 50%) used in the main clinical trials was debatable. Therefore the applicant was requested by the CHMP to submit additional analyses using different cut-off values regarding the clinically significant stenosis; when using 60, 70, 75 and 80% cut-off values, the results were similar to those using the 50% cut-off value.

Discussion on clinical efficacy

The efficacy of Gadofosveset was shown in two phase II dose-finding studies and four main phase III studies. In patients with known or suspected abdominal or limb vascular disease Gadofosveset enhanced MRA was more accurate than unenhanced MRA for detection of stenosis greater than 50%. Gadofosveset enhanced MRA showed a statistically significant improvement in diagnostic efficacy (sensitivity, specificity, and overall accuracy) compared to unenhanced MRA. The increase in sensitivity in AIOD and renal arteries ranged from 14-28%. All blinded readers (12/12) showed clinically and statistically significant improvement in specificity. In the pedal study there was no consistent improvement in sensitivity observed with Gadofosveset reflecting the well-recognized problem of precontrast MR imaging in low flow vessels. The increase in specificity for post-contrast MRA compared to pre-contrast MRA (21-35%) could, however, be translated into an increased ability to correctly identify normal pedal vessels.

Regarding the impact on therapeutic decisions, in case of false positive MRA results, most often contrast XRA is carried out before the revascularisation intervention; if no signs/symptoms of high grade stenosis are seen the patient will not undergo revascularisation intervention. In false negative cases, if there are clinical symptoms and / or functional tests are positive, a further diagnostic procedure is indicated. It was clear that a higher number of patients would undergo XRA procedure based on unenhanced MRA alone compared to MS-325 enhanced MRA. Thus, use of Gadofosveset enhanced MRA will result in substantial reduction in number of patients who would be exposed to the

known risks of XRA.

A limitation of the Gadofosveset clinical studies was that no head to head comparisons with currently available MRA contrast agents were carried out. This was due to the fact that no extracellular agent had a European-wide approval for MRA during development of Gadofosveset [only gadoterate meglumine (Dotarem) and gadobutol (Gadovist) had received approval for MRA in a number of European countries]. The overall sensitivity and specificity for both post-contrast and pre-contrast MRA in the Gadofosveset studies were lower than those typically reported in the MR literature, where values over 90% are given. This could have been a result of: (a) complete blinding of the radiologists from any clinical data on the patients, (b) use of independent radiologists to read the MRA and XRA data, (c) use of the intent-to-treat analysis that considers all un-interpretable vessels as inaccurate rather than excluding them, and (d) the use of multiple scanner platforms in multiple sites. All of these features potentially decreased the accuracy, sensitivity and specificity of Gadofosveset. Future studies will therefore be required to claim for any specific features of Gadofosveset compared to the existing contrast agents.

No clinical information was available about the repeated use of Gadofosveset. No studies were carried out in pediatric or adolescent patients.

The initial requested indication; "contrast-enhanced magnetic resonance angiography in patients with suspected or known vascular disease" was too broad. Diagnostic properties in the cerebrovascular, the coronary vascular, arterial pulmonary angiography or venous angiography systems were not investigated and the use of Gadofosveset should therefore be restricted to the abdominal and limb vascular systems.

Standard design features relevant for development of diagnostic tests and pharmaceuticals were included in the development plan according to regulatory guidelines.

Clinical safety

Data from 18 clinical studies were included in the safety data-base. These studies included seven clinical phase I/II pharmacological studies, two dose ranging phase II studies performed in patients with vascular disease, four pivotal phase III studies performed in different vascular territories representating different flow states and five additional feasibility studies in other indications included into the safety section but not into the efficacy evaluation. The studies included 1,438 subjects administered MS-325 and 79 subjects administered placebo. All subjects who received at least one dose of study medication (including placebo in the placebo-controlled studies) were included in the data-base.

- Patient exposure and demographics

A total of 1,438 subjects (117 healthy volunteers, 1,203 patients with known or suspected vascular disease, and 118 other patients) received MS-325 Injection. Among the 1,321 patients who received MS-325 Injection the mean age was 62.8 years (range: 21 – 91 years), 65.5% were men and 79.9% were Caucasian.

One hundred and seventeen healthy volunteers were administered MS-325 in doses ranging from 0.01 to 0.15 mmol/kg (5 fold greater dose than the proposed clinical dose). Thirty healthy volunteers and 49 patients were administered placebo.

MS-325 was administered to vascular disease patients in doses ranging from 0.005 mmol/kg to 0.100 mmol/kg. Safety of the single bolus *iv* injection of 0.03 mmol/kg was evaluated in 767 patients.

In addition to the dose ranging and main phase trials in vascular disease patients, trials were undertaken in patients with vascular disease on concomitant warfarin therapy (MS-325-06), mild to severe renal impairment (MS-325-07) and end-stage dialysis (MS-325-18), hepatic impairment (MS-325-16), breast cancer (MS-325-05), and coronary artery disease (MS-325-04/04A, MS-325-10, and

MS-325–11, all included in the safety data-base). Sixty-three % of patients had a history of hypertension and approximately 53% had known coronary artery disease. Twenty-four % had a history of hypercholesterolemia. Diabetic patients accounted for approximately 33% of the overall patient population.

Due to the substantial incidence of cardiovascular disease in the MS-325 clinical trials, more than 95% of patients were receiving concomitant treatment of cardiovascular disease. For example, the most common concomitant medications were aspirin taken by 54% of patients and acetaminophen (8.4%), atenolol (15%) and metoprolol (8%), hydrochlorothiazide (11.2%) and furosemide (10.1%), and lisinopril (10.3%), ramipril and enalapril (5% each). Atorvastatin and simvastatin were each taken by 14% of patients. Diabetic medications included glibenclamide (8.1%), insulin (6.0%), metformin (5.3%), and glipizide (4.0%). Other medications included nitroglycerin taken by 9.3% of patients and digoxin in 4.0%.

- Adverse events

Adverse events were monitored from baseline to the end of the study follow up, usually 72 hours after injection. In special pharmacological studies follow up was extended up to 14 days.

Overall, the 1,321 MS-325-treated patients experienced 1,292 AEs, including 769 treatment-related AEs (i.e., those considered by the investigator to be possibly or probably related to treatment).

Of all AEs, 80.2% were mild, 17.0% were moderate, and 2.8% were severe. Of the treatment-related AEs, 81.4% were mild, 16.5% were moderate, and 2.1% were severe.

The most common treatment-related AEs are given in the Table 8 below; their occurrence was dose-dependent.

Table 8: Number and percent of patients who experienced the most common treatment-related adverse events (events reported by > 1% of all patients) by dose group and preferred term for all patients who received Gadofosveset

Preferred Term	**Placebo (N = 49)**	**Dose Group (mmol/kg)**				**All Doses Combined (N = 1,321)**
		< 0.03 (N = 95)	**0.03 (N = 767)**	**0.05 (N = 348)**	**> 0.05 (N = 111)**	
Any treatment-related AE	16 (32.7)	14 (14.7)	176 (22.9)	150 (43.1)	75 (67.6)	415 (31.4)
Pruritus NOS	1 (2.0)	1 (1.1)	34 (4.4)	39 (11.2)	20 (18.0)	94 (7.1)
Paresthesia	1 (2.0)	0 (0.0)	20 (2.6)	39 (11.2)	19 (17.1)	78 (5.9)
Headache NOS	2 (4.1)	1 (1.1)	17 (2.2)	13 (3.7)	3 (2.7)	34 (2.6)
Nausea	0 (0.0)	1 (1.1)	29 (3.8)	25 (7.2)	5 (4.5)	60 (4.5)
Vasodilatation	0 (0.0)	1 (1.1)	22 (2.9)	19 (5.5)	22 (19.8)	64 (4.8)
Burning sensation NOS	0 (0.0)	0 (0.0)	15 (2.0)	28 (8.0)	17 (15.3)	60 (4.5)
Dysgeusia	6 (12.2)	2 (2.1)	17 (2.2)	20 (5.7)	5 (4.5)	44 (3.3)
Feeling cold	0 (0.0)	2 (2.1)	5 (0.7)	10 (2.9)	0 (0.0)	17 (1.3)

N is the total number of patients in the dose group; % is based on N. Causality assessed by the clinical investigator.

The uncommon (≥ 1: 1000, < 1: 100) adverse reactions included vomiting NOS, retching, diarrhoea NOS, abdominal discomfort, abdominal pain NOS, dry mouth, flatulence, hypoesthesia lips, salivary hypersecretion, nasopharyngitis, hypersensitivity NOS, hyperglycaemia NOS, hypocalcaemia, anxiety NEC, ageusia, dizziness (excl. vertigo), tremor, hypoesthesia, parosmia, lacrimation increased, atrioventricular block first degree, tachycardia NOS, hypertension NOS, phlebitis NOS, dyspnea NOS, urticaria NOS, erythema, rash NOS, sweating increased, muscle cramps, muscle spasms, neck pain,

pain in limb, hematuria, microalbuminuria, glycosuria, pain NOS, chest pain, fatigue, feeling abnormal, groin pain, injection site pain, injection site coldness, injection site erythema, electrocardiogram QT prolonged. The rare (< 1:1000) adverse reactions included cellulitis, urinary tract infection NOS, hypokalemia, hyperkalemia, electrolyte imbalance, hypernatremia, appetite decreased NOS, hallucination NOS, abnormal dreams, muscle contractions involuntary, asthenopia, abnormal sensation in eye, ear pain, cardiac flutter, myocardial ischaemia, atrial fibrillation, bradycardia NOS, palpitations, arteriosclerosis, hypotension NOS, respiratory depression, cough, dyspepsia, pharyngolaryngeal pain, clamminess, muscle tightness, sensation of heaviness, micturition urgency, renal pain, urinary frequency, pelvic pain NOS, pyrexia, rigours, weakness, chest pressure sensation, feeling hot, injection site thrombosis, injection site bruising, injection site inflammation, injection site burning, injection site extravasation, injection site haemorrhage, injection site pruritus, sensation of pressure NOS, electrocardiogram ST segment depression, electrocardiogram T wave amplitude decreased, electrocardiogram abnormal NOS, anaphylactoid reaction, phantom limb pain.

The only severe AEs experienced by more than one patient were pruritus NOS (0.3%), burning sensation NOS and headache NOS (0.2%), and myocardial infarction and syncope (0.2%). The only severe event experienced by more than one patient receiving 0.03 mmol/kg MS-325 was headache NOS (0.3%).

The majority of AEs had an onset shortly after drug administration (often within 5 minutes). Headache NOS, injection site bruising, and venipuncture site bruise typically had longer times to onset. Most AEs were transient, with more than 50% of all AEs resolving within 1 hour of onset; a few delayed reactions (after hours or days) occurred.

- Serious Adverse Events

Twelve patients experienced a total of 15 SAEs. Two of the SAEs were experienced by patients who received < 0.03 mmol/kg MS-325, five by patients who received 0.03 mmol/kg MS-325 (coronary artery disease, hyperglycemia NOS, gangrene NOS, chest pain and anaphylactoid reaction; this anaphylactoid reaction was considered probably related and was resolved within 5 minutes), four by patients who received 0.05 mmol/kg MS-325, and two by patients who received > 0.05 mmol/kg MS-325. Four SAEs, including 1 death, were considered by the investigator to be possibly or probably related to MS-325. There was no evidence of dose dependency either in the frequency of SAEs either in general or for any particular SAE.

Two patient deaths were reported. One patient received 0.005 mmol/kg MS-325 and died of complications of a myocardial infarction and multisystem organ failure. This was considered unlikely related to MS-325. The other fatal case received 0.07 mmol/kg of MS-325 and with an SAE of arteriosclerosis reported. This event was considered possibly related to MS-325. In addition, one additional patient died during the Study MS-325-18 (which was ongoing at the time of cut-off for this dossier). This death was not considered to be related to administration of MS-325.

- Laboratory findings

There was no evidence of a clinically significant systematic change from baseline in any post-dosing vital signs evaluations for any of the patients who received MS-325

Cardiac safety / ECG

For all patients, standard 12-lead ECG was generally performed before dosing and at three to four time points post-dosing (over 72 hours; for some special population pharmacokinetic studies, several post-dose ECGs were collected up to 14 days post-dose). Analyses of ECG data followed the FDA and EU guidelines.

Gadofosveset administration did not cause any clinically significant changes in heart rate. Although there were statistically significant increases in PR interval and QRS interval at 45 minutes post dosing, these findings were not clearly dose dependent and a relationship in time was not observed. There was

a statistically significant mean increase from baseline (2.7 msec) in QTc (Bazett's method) at 45 minutes post-dosing; the changes at 24 and 72 hours were not statistically significant. 15 subjects out of 1226 patients had QTc increases > 60 milliseconds at any of the three post dosing time points. There was no evidence of a clinically significant increase in the number of patients with QTc > 500 msec after dosing.

In patients who received doses > 0.05 mmol/kg gadofosveset, there was an increase of 8.8 msec (Bazett correction) and 8.5 msec (Fridericia correction) in the QTc at 45 minutes. In the patients who received placebo the change was 4.7 msec. However, analysis of data of holter monitoring where multiple predose ECGs were available showed that between 12 hours and 30 minutes pre dosing, the QTc decreased by 7.6 msec, while it increased by 4.6 msec between 30 and 5 minutes pre dose, demonstrating thus substantial variability of these intervals. These findings have been reflected in SPC (4.4.).

No clinically significant changes in any laboratory parameters were seen after MS-325 injection. Specifically, there was no evidence of a depletion of zinc, calcium, or iron from serum following MS-325 administration. A modest increase in the amount of zinc recovered from urine in the 24 hours after treatment was considered to be of no clinical significance and was lower than that expected with other Gd-based contrast agents.

- Immunological events

There were no findings of concern noted for any of the immunological parameters assessed that were indicative of acute changes (over 3-4 days), nor significant changes indicative of delayed hypersensitivity (over a period of up to 21 days post-administration). Twenty-seven patients (2.0%) had a history of allergy to contrast media or iodine and one patient experienced a mild "allergic reaction" upon administration of MS-325. There were no significant changes in the immunology panel assays for these patients.

Pruritus NOS and paresthesia were the most common AEs and were considered most often by investigators to be possibly or probably related to treatment and also showed an apparent dose-dependent increase in incidence. Burning sensation NOS was also common and was commonly treatment-related.

In the early phase clinical studies, patients reported a transient sensation occurring immediately after injection that was localized to the perineal region. This resolved spontaneously without intervention after a minute or two and was mild in nature in the majority of patients. A panel of immunology experts had reviewed both AE data and the immunological laboratory data available at that time and had concluded that the event did not represent an immunological process.

- Discontinuation due to adverse events

Three patients discontinued a study because of AEs. Two of the patients received 0.05 mmol/kg MS-325, and one received 0.07 mmol/kg. The AEs leading to discontinuation were renal mass NOS, arteriosclerosis and myocardial infarction; of these events, only arteriosclerosis was considered possibly related to MS-325.

- Discussion on clinical safety

Gadofosveset was in general safe and well tolerated. The most common treatment-related AEs were pruritus, paresthesia and burning sensation, and their occurrence was dose-dependent. Other common AEs included vasodilatation, headache, and nausea. The AEs were usually mild, rapid onset and of short duration.

There was no evidence that patients with renal impairment or moderate hepatic impirement would be at increased risk for experiencing AEs or clinical laboratory abnormalities. However, in patients with

more severely impaired renal function (clearance < 20 ml/min) who are not supported by routine dialysis, the benefits must be weighed very carefully against the risks. In a clinical trial it was shown that gadofosveset can be effectively removed from the body by dialysis using high flux filters.These findings have been reflected in SPC (4.4.).

Additional adverse reactions related to protein binding were considered possible, and thus interaction studies were performed in-vitro. In-vivo only one interaction study was conducted with warfarin. There was no evidence of significant drug-drug interactions. The rates of occurrence of AEs were not altered by the concomitant administration of gadofosvet or other albumin-binding drugs, such as warfarin, although the number of subjects evaluated was too low for being conclusive. These findings have been reflected in SPC (4.5.).

Mild QT-prolongation was reported during the Gadofosveset treatment, similar to other Gd containing contrast agents. Elevated levels of gadofosveset may increase the possibility of QT prolongation, there was an increase of 8.8 msec in patients who received doses > 0.05 mmol/kg gadofosveset. These findings have been reflected in SPC (4.4.).

A major safety concern in the development of this class of agents is the possible release of free Gd in vivo, as this heavy metal is extremely toxic. Administration of Gadofosveset did not have any clinically important effects on plasma and urinary levels of metals such as calcium, iron, and zinc.

The safety of repeated doses of Gadofosveset was not studied in clinical trials. Clinical studies on pediatric patients were not carried out. These facts have been reflected in SPC (4.2.).

As with other diagnostic procedures involving MRI contrast agents, there are several safety precautions to be followed. These are reflected in SPC (4.4).

3.4.1. Overall conclusions, benefit/risk assessment and recommendation

Quality
The pharmaceutical documentation showed the quality is acceptable and minor quality issues will be resolved as Follow-up Measures.

Non-clinical pharmacology and toxicology

Preclinical pharmacology studies showed, that MS-325, a new gadolinium-based MRI contrast medium was efficacious in enhancing various vascular beds and in detecting structural abnormalities at the intended single dose of 0.03 mmol/kg.

Preclinical data revealed no special hazard for humans based on conventional studies of safety pharmacology, acute toxicity, local tolerance, contact-sensitising potential, and genotoxicity.
No carcinogenicity studies were performed, since Vasovist is intended to be used for diagnostic investigations which will be carried out only once.

Repeated-dose toxicity studies revealed vacuolation of the tubular cells of the kidneys, with strong evidence for reversibility of the effect. No functional impairment was observed and electron microscopic investigations of the rat kidneys indicated that the observed vacuolation was primarily a storage phenomenon. Effects were of higher severity in rats than in monkeys, probably because of the higher renal clearance in rats. In monkeys, no renal effects were observed after single use even at a dose 100-times higher than the clinical dose.

In rabbit reproduction toxicity studies an increased number of early resorptions and a slight but significant increase in the number of foetal anomalies were observed at dosages at which no or slight maternal toxicity was observed.

Overall, the preclinical findings suggested that Gadofosfeset does not pose a significant hazard to humans at therapeutic doses.

Efficacy

Standard design features relevant for development of diagnostic tests and pharmaceuticals were included in the development plan according to regulatory guidelines.

The results from two dose-finding phase II studies and from four main phase III studies performed in different vascular territories representing different flow states showed, that Gadofosveset improved "structure delineation," i.e. delineation of normal vascular anatomy and vascular pathology such as stenotic or aneurysmatic vessels by means of contrast-enhancement in patients with known or suspected vascular disease of the aortoiliac and renal region. Gadofosveset enhanced MRA was more accurate when compared with unenhanced MRA for assessment of arterial stenosis greater than 50%. Gadofosveset enhanced MRA showed a statistically significant improvement in diagnostic efficacy (sensitivity, specificity, and overall accuracy) compared to unenhanced MRA. In the pedal artery study the specificity increased for post-contrast MRA. The benefit was demonstrated regarding technical efficacy, diagnostic efficacy, diagnostic thinking efficacy and therapeutic decision making compared to unenhanced MRA in the abdominal and peripheral vascular system using the standard procedure X-ray angiography as the standard of reference.

Safety

Gadofosveset was well tolerated in general. The most common side effects were pruritus, paresthesia, headache NOS (not other specified), nausea, vasodilatation, burning sensation NOS and dysgeusia; these occurred generally soon after dose administration, were transient, mild or moderate in intensity, and nearly always resolved without intervention. Mild QT-prolongation was reported, similar to other Gd containing contrast agents. Safety of repeated doses of Gadofosveset or safety in patients < 18 years of age were not studied in clinical trials.

Benefit/risk assessment

Benefit of a single administration of Gadofosveset with MRA, using a dose of 0.03 mmol/kg, was established in terms of technical and diagnostic efficacy, diagnostic thinking efficacy and therapeutic decision making compared to unenhanced MRA, in the abdominal and peripheral vascular system using the standard procedure X-ray angiography as a standard of reference. Gadofosveset improved "structure delineation," i.e. delineation of normal vascular anatomy and vascular pathology such as stenotic or aneurysmatic vessels, in these patients. The initial requested indication, "contrast-enhanced magnetic resonance angiography in patients with suspected or known vascular disease", was too broad as assessed by the CHMP, because diagnostic properties in the cerebrovascular, coronary vascular, arterial pulmonary angiography or venous angiography systems had not been investigated, and the use of Gadofosveset was thus restricted to the abdominal and limb vascular systems. The clinical safety profile of Gadofosveset did not raise major concerns; the most common side effects occurred generally soon after dose administration, were transient, mild or moderate in intensity, and nearly always resolved without intervention. No studies in children or adolescents < 18 years old were conducted. No studies on repeated doses were conducted.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered that the benefit/risk ratio in contrast-enhanced magnetic resonance angiography for visualization of abdominal or limb vessels in patients with suspected or known vascular disease of Vasovist was favourable and therefore recommended the granting of the marketing authorisation.