

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**21-711**

**CLINICAL PHARMACOLOGY AND  
BIOPHARMACEUTICS REVIEW(S)**

<i>NDA</i>	21-711	<i>Submission Date(s)</i>	June 30, 2008
<i>Brand Name</i>	Vasovist™ Injection		
<i>Generic Name</i>	Gadofosveset trisodium		
<i>Reviewer</i>	Christy S. John, Ph.D		
<i>Team Leader</i>	Young Moon Choi, Ph.D.		
<i>Division Director</i>	NAM Atiqur Rahman, Ph.D.		
<i>OCP Division</i>	V		
<i>ORM Division</i>	Division of Medical Imaging and Hematology Drug Products		
<i>Sponsor</i>	EPIX Medical Inc		
<i>Relevant IND(s)</i>	IND 51,172		
<i>Submission Type</i>	Re-submission		
<i>Dose and Route of administration</i>	0.03 mmol/kg, Intravenous injection		
<i>Indication</i>	<p>VASOVIST Injection is a gadolinium-based blood pool contrast agent indicated for use with magnetic resonance angiography (MRA) to evaluate aortoiliac occlusive disease (AIOD) in adults with known or suspected peripheral vascular disease.</p>		

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## 1 Executive Summary

Vasovist Injection (also known as gadofosveset trisodium) is a new molecular entity, a gadolinium based contrast agent. Vasovist is developed for magnetic resonance angiography (MRA), b(4)

in adults with known vascular disease. Original NDA 21-711 was submitted on December 12, 2003. Clinical pharmacology review was filed in DFS on December 3, 2004. From a clinical pharmacology perspective, the original submission was acceptable. The submission, however, was not approved due to the lack of substantial evidence that sensitivity and specificity of vasovist enhanced MRA to that of non-enhanced MRA (Please refer to the approvable letter dated 1/12/2005 in DFS).

On June 30, 2008, the applicant re-submitted the application after a blinded re-read of Phase III results of the original NDA to confirm the diagnostic performance of vasovist in patients with aortoiliac occlusive disease (AIOD). The re-read results are under review by clinical and statistical team.

Vasovist is a unique agent designed for MRA. The biological half-life of this agent is 16-18 hours as compared to 1-2 hours for other approved gadolinium contrast agents. The elimination half-life of vasovist in patients with moderate and severe renal impairment is 49 and 69 hours, respectively. This long half-life raises concerns about the in-vivo stability (dissociation of gadolinium ion from gadolinium chelate) of the chelate in patients with moderate and severe renal impairment.

In 2007, the Agency issued a "Black Box Warning" for all gadolinium based contrast agents carrying a risk for nephrogenic systemic fibrosis (NSF) for patients with severe renal impairment and renal insufficiency of any severity due to the hepato-renal syndrome or in the perioperative liver transplantation period. The label recommended to avoid use of gadolinium based contrast agents in these patient populations unless the diagnostic information is essential and can not be obtained with non-contrast enhanced magnetic resonance imaging (MRI).

Due to high risk of NSF in patients with renal impairment after administration of gadolinium containing contrast agents, limited safety data, long-half life of the agent, and higher exposure in patients with renal impairment, we recommend that applicant conduct a safety study exploring lower dose in patients with moderate and severe renal impairment. The moderate renal impaired patients achieved exposure which is two-fold higher than the exposure in normal renal function patients and the clinical study demonstrated no clear dose-response at the dose levels tested. Therefore, exploration of a lower dose in renal impaired patients is appropriate. We recommend that this concern be expressed in label.

The applicant did not conduct thorough QT study, however, ECG monitoring was performed in patients and healthy volunteers following vasovist administration. The mean QTc change at 45 minute after administration of the proposed dose of 0.03 mmole/kg was less than 10 msec. Forty two out of 702 (about 6 %) subjects showed absolute increase of QTc over 30 msec from baseline. We recommend that this concern be expressed in label.

Clinical pharmacology team reviewed the proposed labeling following a PLR format. The labeling changes have been recommended.

**1.1 Recommendations:**

From the clinical pharmacology perspective, the application, NDA 21-711 (Vasovist Injection) is acceptable provided that the applicant and the agency mutually agree on the labeling language. For the agency's specific labeling recommendation, please refer to the section 3 detailed labeling recommendation of the present review (Page 6).

**1.2 Phase IV Commitments: N/A**

**1.3 Summary of Clinical Pharmacology Findings (from Original Review):**

The Office of Clinical Pharmacology reviewed NDA 21-711 submitted on December 12, 2003.

The following were brief findings noted:

**Mechanism of action:** Vasovist binds to plasma proteins reversibly. The plasma protein binding renders Vasovist a favorable attribute for imaging of the vascular system with magnetic resonance imaging. In vivo human and pre-clinical protein binding extends the residence time of the drug in vascular space, thereby extending the imaging period. The protein binding of Vasovist increases the relaxivity greater than five-fold in plasma solution (compared to that in aqueous solution).

**Pharmacodynamics:** The pharmacodynamics of Vasovist was determined by measuring the ex vivo plasma 1/T1. The high relaxivity of Vasovist is attributed to its protein binding. With increasing dose, 1/T1 increased and remained elevated throughout the imaging window of one hour post-dose.

**Distribution, Metabolism, and Excretion:** A study 0.03 mmol/kg in healthy volunteers at showed that the plasma concentration-time profile conforms to an open two-compartment model. Vasovist concentration declined rapidly during distribution phase, (t<sub>1/2α</sub>) (0.48±11 hours) and more slowly during disposition phase, (t<sub>1/2β</sub>) (16.3±2.6 hours). The mean plasma concentration at one hour dosing was 56% of the concentration at 3 minutes after injection. The mean total clearance was 6.6±1.0 mL/h/kg (i.e. 7.7 mL/min for a 70 kg person).

Total clearance, Cl(t), and renal clearance Cl(r), values increased with the dose of Vasovist. Total clearance and renal clearance are similar since urinary excretion is the main route of elimination of Vasovist. Removal of Vasovist from circulation appears more efficient at higher plasma concentrations, as reflected in the values of total and renal clearance over the entire dose range. The mean terminal plasma half-life of MS-325 did not vary remarkably with dose (range: 13.4 to 18.0 hrs).

The steady-state volume of distribution, V(ss), tended to increase slightly with an increase in dose above 0.05 mmol/kg. The V(ss) (0.016 L/kg at 0.03 mmol/kg) indicates that MS-325 is restricted to the extracellular space (CSR-MS-325-16).

**Dose finding study:** In a dose finding study (Phase II (safety and clinical efficacy study)) three dose groups (0.01 mmol/kg, 0.03 mmol/kg, and 0.05 mmol/kg) in patients with carotid and peripheral arteries stenosis were studied. No clear dose response was observed.

Table I. Determining disease state for all three regions, i.e., carotid, iliac, and femoral arteries, combined after 5(±4) minute post- MS-325 IV administration

Sensitivity %	88	86	89	64	100	76	75	80	79
Specificity %	100	67	75	100	85	88	82	75	79
Accuracy %	91	80	85	78	92	87	78	77	75
Kappa	0.79	0.52	NA	0.58	0.83	NA	0.57	0.55	NA

NA= not available

\*Comb = left and right sides were combined in a tertiary analysis in order to better summarize the data

**Effects on Electrocardiography:** The sponsor did not conduct thorough QT study, however, ECG monitoring was performed in clinical trials at screening and up to 21 days following gadofosveset dosing in patients and healthy volunteers. QTc was derived using the Bazett's formula. At the recommended clinical dose of gadofosveset 0.03 mmol/kg, the mean increase from baseline in QTc was similar between gadofosveset and placebo subjects. The mean increase in QTc at the 45 minute time point at a dose of 0.03 mmol/kg was 2.8 msec (N=702; p< 0.05), compared to 3.2 msec in placebo subjects (N=38; p=NS). QTc changes were not associated with any adverse events, including arrhythmias.

The number of subjects with changes from baseline QTc of 30-60 msec at 45 minutes were: 1/38 (2.6%) in placebo subjects, 1/43 (2.3%) at the 0.005 mmol/kg dose, 1/48 (2.1%) at 0.010 mmol/kg, 39/702 (5.6%) at 0.03 mmol/kg, 9/219 (4.1%) at 0.05 mmol/kg, 1/37 (2.7%) at 0.07 mmol/kg, and 7/70 (10%) at 0.1 mmol/kg. Changes from baseline QTc of >60 msec at 45 minutes were only observed at gadofosveset doses of 0.03 mmol/kg (3/702; 0.4%), 0.05 mmol/kg (1/219; 0.5%), and 0.1 mmol/kg (1/70; 1.4%).

**Drug-Drug interactions:** Warfarin may be used in subjects with vascular disease requiring diagnostic MR imaging, and because both warfarin and vasovist bind to human serum albumin. A study was undertaken in subjects with arterial vascular disease to assess potential PK/PD interactions of Vasovist and warfarin. The results showed that the PK profile of vasovist was unaltered in subjects on concurrent warfarin therapy as compared to those who did not receive warfarin. The concentrations in plasma and plasma unbound fraction were nearly super-imposable in the presence or absence of warfarin.

**Special populations:** There is no difference in pharmacokinetics of vasovist between male and female. There is also no significant difference in kinetics between adults and elderly 65 years and over. Adjustment of dose in geriatric population is not necessary.

**Renal insufficiency:** Vasovist is cleared through the kidneys, the pharmacokinetics of vasovist were noticeably affected by renal impairment following a single 0.05 mmol/kg dose. Vasovist plasma concentrations were studied following a single IV dose of Vasovist (0.05 mmol/kg) in subjects with varying degrees (mild, moderate and severe) of renal impairment. Subjects were classified based upon creatinine clearance calculated using the Cockcroft-Gault (C-G) equation from baseline serum creatinine values. As renal function decreased, plasma concentration at a given time post-dose increased while the systemic clearance of vasovist decreased. The renal clearance decreased substantially in patients with moderate and severe renal impairment. The exposure (AUC) increased almost two fold in patients with moderate and severe renal impairment. The half-life increased from 19 hrs in normal subjects to 49 hours in patients with moderate renal impairment to 70 hours in patients with severe renal impairment. A prolonged half-life in renal insufficient patients can potentially cause in-vivo dissociation of Gd-fosveset that can potentially lead to free gadolinium ion toxicity and complexation of free ligand with calcium and other metals such as iron, magnesium etc.

*Comment: With Agency's black box warning for all gadolinium agents, the sponsor is obligated to conduct safety study of vasovist in patients with moderate and severe renal impairment.*

**Hepatic impairment:** There were no important differences in the concentration-time profile of vasovist or differences in the plasma protein bound fraction with time in the hepatic impaired subjects and age matched normal volunteers.

## 2. Question Based Review:

For a complete review of clinical pharmacology of vasovist (NDA 21-711) please refer to the review in DFS.

16 Page(s) Withheld

       Trade Secret / Confidential (b4)

✓ Draft Labeling (b4)

       Draft Labeling (b5)

       Deliberative Process (b5)

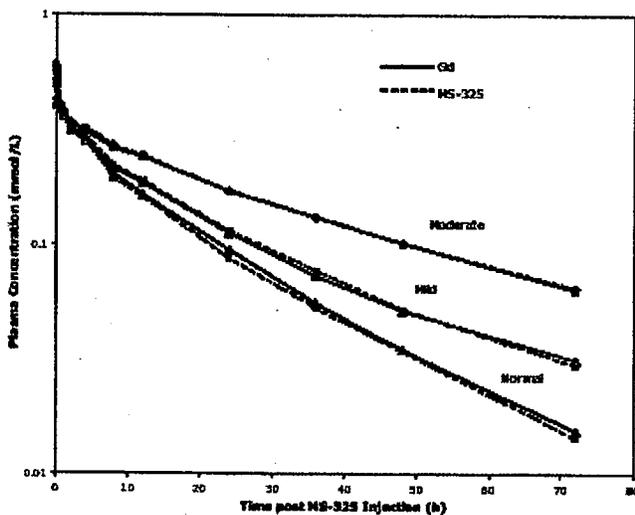
#### 4.2. Renal Insufficiency Study (Study MS-325-07)

##### Pharmacokinetics of MS-325 in subjects with varying degree of renal insufficiency:

Nineteen serial plasma samples, 10 urine samples, and 10 fecal samples were obtained from subjects in the mild and normal groups from Baseline through 14 days post-MS-325 injection. Additional plasma, urine, and fecal samples were collected from subjects in the moderate group at 21 days post-MS-325 injection. MS-325 was assayed in plasma and urine samples by an HPLC-UV method. Plasma, urine, and feces were also assayed for total Gd using an ICP-AES assay. Plasma concentration of non-protein bound (unbound) MS-325 (i.e., concentration in PUF) was also determined using the ICP-AES assay. The total MS-325 concentration-time data were analyzed for PK parameters using an open two-compartment (bi-exponential) model and a non-compartmental method using WinNonlin. Plasma clearance curves are shown in Figure XI. The following parameters were calculated: AUC, C<sub>max</sub>, t(1/2 $\alpha$ ), t(1/2 $\beta$ ), MRT, V(ss), Cl(t), and Cl(r). Cumulative urinary, X(u), and fecal, X(f), excretion were expressed as percent of administered dose.

The PK samples were assayed for total Gd using a validated ICP-AES method. In brief, a 0.5 mL or 0.5 g sample of plasma, PUF, urine, or fecal homogenate was digested on a hot plate with nitric acid and hydrogen peroxide. The amount of Gd was determined with an ICP spectrometer by comparing the emission of the unknown sample to the emission of the external standard solutions. The results were generated as  $\mu\text{g/mL}$ . Concentration as mmol/L was obtained by dividing by the atomic weight of Gd (157.25 g/mol). The limit of quantitation (LOQ) of Gd was 1  $\mu\text{g/mL}$ . A set of calibration standards (five concentrations each in duplicate) and QC samples (three concentrations each in duplicate) were included in each analysis session to generate a standard curve and to assess assay performance. One blank was run at the beginning and end of each run to check for contamination.

Figure XI. Plasma clearance of MS-325 with time in normal volunteers and patients with mild and moderate renal impairment



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The calculated PK parameters for renal impaired subjects is shown in Table XI. The renal clearance decreased substantially in patients with moderate and severe renal impairment. The exposure (AUC) increased almost two fold in patients with moderate and severe renal impairment. The half-life increased from 19 hrs in normal subjects to 49 hours in patients with moderate renal impairment to 70 hours in patients with severe renal

impairment.

Table XI. PK profile of vasovist in renal impaired patients

Parameter	Normal	Mild	Moderate	Severe
C <sub>max</sub> , mmol/L	0.71 (26)	0.57 (16)	0.61 (26)	0.67 (21)
AUC(0-inf), mmol* <i>h</i> /L	7.12 (12)	7.52 (28)	12.5 (30)	16.1 (23)
V(ss), L/kg	0.16 (10)	0.17 (12)	0.19 (14)	0.18 (11)
t(1/2 <sub>term</sub> ), h	18.9 (14)	22.5 (40)	49.0 (32)	69.5 (43)
Cl(t), mL/h/kg	7.1 (12)	7.0 (29)	4.1 (36)	3.0 (37)
Cl(r), mL/h/kg	5.3 (17)	5.7 (27)	3.0 (34)	2.2 (38)
X(u), % †	74.2 (12)	80.7 (9)	69.1 (11)	65.8 (12)
X(f), % †	6.5 (53)	7.8 (34)	8.5 (58)	13.3 (46)

Note: Renal function was classified using the C-G method: creatinine clearance normal >80 mL/minute; mild impairment >50-80 mL/minute; moderate impairment 30-50 mL/minute; and severe impairment <30 mL/minute. Urine and feces were collected over a 168-hour interval. Source: CSR MS-325-07, Section 11.4.2.2

The determination of PK parameters using two different analytical methods (HPLC-UV assay and ICP-AES) was very comparable (Table XII).

Table XII. Comparison of PK parameters determined by two different analytical method

Parameter	MS-325 by HPLC-UV Assay			Gd by ICP-AES Assay		
	Group			Group		
	Normal Mean, %CV	Mild Mean, %CV	Moderate Mean, %CV	Normal Mean, %CV	Mild Mean, %CV	Moderate Mean, %CV
C <sub>max</sub> , mmol/L	0.690, 24	0.577, 20	0.663, 23	0.699, 23	0.556, 19	0.650, 23
T <sub>max</sub> , hr*	0.0170, 88	0.0830, 55	0.0170, 85	0.0170, 74	0.0830, 57	0.0170, 74
V(ss), mL/kg	165, 9	177, 19	199, 18	161, 10	173, 11	189, 13
t(1/2 <sub>alpha</sub> ), hr	1.41, 76	4.87, 96	8.42, 97	1.22, 86	3.63, 99	8.21, 105
t(1/2 <sub>beta</sub> ), hr	17.2, 17	31.3, 75	64.9, 64	17.0, 14	26.4, 61	54.2, 48
t(1/2 <sub>term</sub> ), hr	19.2, 16	34.8, 85	69.3, 56	19.0, 13	26.4, 44	61.4, 47
MRT, hr	22.8, 15	35.0, 60	68.2, 45	23.0, 13	31.8, 45	62.5, 39
AUC(0-inf), mmol* <i>h</i> /L	6.89, 12	9.49, 45	16.7, 35	7.16, 12	9.08, 41	16.3, 35
Cl(t), mL/h/kg	7.36, 12	6.15, 40	3.44, 42	7.07, 12	6.29, 37	3.51, 40
Cl(r), mL/h/kg	5.59, 15	5.38, 40	2.66, 49	5.39, 17	5.05, 38	2.60, 40
X(u), %	74.2, 12	84.7, 8	64.5, 21	74.6, 11	77.9, 11	67.5, 12
X(f), %	Not Done	Not Done	Not Done	6.38, 53	8.27, 32	11.6, 49

\* The values presented for T<sub>max</sub> are the median and percent coefficient of variation (%CV).

Note: Subject group classification based on per-protocol criteria for renal function.

Note: One subject in the moderate group was excluded from the mean Gd calculation of X(f) due to no samples recorded from 24 to 120 hours post-dose. One subject in the moderate group was excluded from mean MS-325 calculation of Cl(r); three subjects in the moderate group were excluded from the mean MS-325 calculation of X(u) and one subject in the normal group was excluded from the mean MS-325 calculation of X(f) due to many concentration points not reportable (interference).

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**CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW**  
(November 1, 2005)

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<b><u>NDA:</u></b>	21-711
<b><u>Submission Date:</u></b>	January 5, 2005
<b><u>Category:</u></b>	1S
<b><u>Proposed Brand Name:</u></b>	Vasovist (gadofosveset trisodium) injection
<b><u>Formulations:</u></b>	0.250 mmol/mL (244 mg/mL) solution for injection
<b><u>Route of Administration:</u></b>	Intravenous
<b><u>Proposed Dose:</u></b>	0.03 mmol/kg
<b><u>Proposed Indication:</u></b>	Contrast agent for magnetic resonance angiography ↓ in adults
<b><u>Sponsor:</u></b>	Epix Medical, Inc. 161 First Street Cambridge, MA 02142
<b><u>Type of Submission:</u></b>	Response to Agency's Request
<b><u>Reviewer:</u></b>	Christy S. John, Ph.D.
<b><u>Team Leader:</u></b>	Young Moon Choi, Ph.D.
<b><u>Received for Review:</u></b>	January 6, 2005
<b><u>Date of Review:</u></b>	November 1, 2005

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In the original OCPB review, the reviewer had asked the sponsor to demonstrate the in-vivo stability of Vasovist in the patients with renal insufficiency by comparing the amount of zinc-fosveset and calcium-fosveset in the urine collected as compared to healthy volunteers. This request is based on:

- (1) Transmetallation and increased excretion of zinc-fosveset corresponds directly to the in-vivo stability of the Gd complex;

- (2) It is expected that a Gd complex with longer biological half-life may have higher potential of transmetallation compared to those with shorter half-lives. It should be noted that the half-life of MS-325 in subjects with normal renal function (16 hours) is much longer than other approved Gd containing MR agents (1-2 hours). Furthermore, in renally impaired patients, the elimination half-life of MS-325 appeared 49-70 hours.

The sponsor sent a response to the Agency on January 5, 2005.

The sponsor stated that they had not studied zinc parameters as the Agency had requested data from them in late Phase III studies. Therefore specific zinc data was not available. However, the sponsor demonstrated in vivo stability as follows:

- (1) The sponsor states that in healthy volunteers, the urinary excretion of MS-325 up to 120 hours post dose showed the presence of only one gadolinium-containing species, which was identified as intact gadofosveset;
- (2) In addition, the sponsor compared Zn excretion in urine after administration of MS-325 and that after administration of Optimark, an approved product. It appeared that in healthy volunteers from the MS-325-16 study, there is relatively small increase (<300 µg/24h) in zinc excretion during the first 24 hours following MS-325 administration compared to 8000 µg of Zn excretion in a much shorter period of time following Optimark administration.
- (3) Correlation of total gadolinium vs. gadofosveset levels in clinical blood samples including those from renal patients shows that there is no evidence for biotransformation of MS-325. The best-fit line has slopes of close to unity indicating all gadolinium is accounted for as gadofosveset.
- (4) In combination with the higher thermodynamic stability of MS-325 compared to other approved products, it can be concluded that increased zinc excretion following MS-325 administration may not be an issue in renal-impaired patients.

In conclusion, given the fact that weaker chelate have much higher amount of zinc excreted in short period of time and that in healthy volunteers there was not substantial increase in zinc excretion of Vasovist, zinc excretion following MS-325 administration may not be an issue.

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**CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS  
REVIEW  
(DRAFT March 22, 2004)**

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<b><u>NDA:</u></b>	21-711
<b><u>Submission Date:</u></b>	December 12, 2003 February 15, 2004
<b><u>Category:</u></b>	1S
<b><u>Proposed Brand Name:</u></b>	Vasovist (gadofosveset trisodium) injection
<b><u>Formulations:</u></b>	0.250 mmol/mL (244 mg/mL) solution for injection
<b><u>Route of Administration:</u></b>	Intravenous
<b><u>Proposed Dose:</u></b>	0.03 mmol/kg
<b><u>Proposed Indication:</u></b>	Contrast agent for magnetic resonance angiography [REDACTED] in adults
<b><u>Sponsor:</u></b>	Epix Medical, Inc.
<b><u>Type of Submission:</u></b>	Original NDA
<b><u>Reviewer:</u></b>	Christy S. John, Ph.D.
<b><u>Team Leader:</u></b>	Young Moon Choi, Ph.D.
<b><u>Dates of Review:</u></b>	Received for Review: January 7, 2004 First Draft: August 9, 2004 Second Draft: December 1, 2004

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## 1. EXECUTIVE SUMMARY:

### 1.1. RECOMMENDATIONS:

- ◆ The Office of Clinical Pharmacology and Biopharmaceutics / Division of Pharmaceutical Evaluation II (OCPB/DPE-II) has reviewed NDA # 21-711 submitted on December 12, 2003. OCPB finds this application acceptable from a clinical pharmacology and biopharmaceutics perspective provided that the sponsor demonstrate the in-vivo stability of Vasovist in the patients with renal insufficiency by comparing the amount of zinc-fosveset and calcium-fosveset in the urine collected as compared to healthy volunteers. In addition, we recommend that total calcium and free calcium ion concentration in the plasma be studied.

This recommendation is based on the following findings:

This reviewer appreciates that gadolinium-fosveset is relatively stable in vivo as demonstrated by comparing the ratios of gadolinium-fosveset and gadolinium ion concentrations in human urine. Nevertheless, the potential of in-vivo dissociation of gadolinium cannot be ruled out completely as evidenced by increased zinc-fosveset excretion in urine after injection of Vasovist (See Table XX in this review on Page 57). Also, the total recovery of gadolinium-fosveset after injection of Vasovist was incomplete (average 83.7% in urine and 4.7% in feces). Furthermore, the extent of Gd dissociation may be greater in patients with renal insufficiency as the terminal half-life of Vasovist is prolonged significantly. As a reference point, there was a five fold increase in zinc excretion in urine (a measure of in-vivo dissociation of Gd) after injection of another approved Gd-containing chelate in renal patients. In this case the elimination half-life increased four fold as compared to normal patients. For Vasovist, the elimination half-life increased up to 49-70 hours depending on degree of renal impairment compared to elimination half-life of 16 hours in normal healthy subjects.

In vivo dissociation of Gd-fosveset may lead to the complexation of free ligand with calcium, magnesium, zinc and iron etc. which may have clinical consequences (such as gadolinium ion toxicity and hypocalcemia). It should be noted that during the review process, the sponsor was asked on 8/30/2004 to provide zinc data. The sponsor reported that they do not have such data.

This reviewer makes the following other key observations:

- ◆ No clear dose response was observed in a dose finding study (Phase II (safety and clinical efficacy study) CSR-MS-325-02). Three dose groups (0.01 mmol/kg, 0.03 mmol/kg, and 0.05 mmol/kg) in patients with carotid and peripheral arteries stenosis were studied, overall there was no significant dose dependent increase in sensitivity, specificity and accuracy. The sponsor selected 0.03 mmol/kg dose for further study **based on the limited data (see the sponsor's rationale for the dose selection on Page 9 of this review).**

- ◆ In renal impaired patients, the renal clearance decreased substantially in patients with moderate and severe renal impairment. The exposure (AUC) increased almost two fold in patients with moderate and severe renal impairment. The half-life increased from 19 hrs in normal subjects to 49 hours in patients with moderate renal impairment and, to 70 hours in patients with severe renal impairment. This increased half-life, slow clearance, and increased systemic exposure may warrant dose reduction from a safety perspective. However, from the efficacy perspective, further dose reduction from 0.03 mmol/kg dose is not warranted.

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- ◆ The mean QTc values did not show an appreciable increase as compared to the placebo group. The placebo and the test group did show mean QTc increase of greater than 10 msec in some patients. A label warning about Vasovist effect on QTc is warranted.
- ◆ The label should be revised based upon the mutual agreement between the sponsor and the Agency.

#### 1.2. PHASE IV COMMITMENTS:

None

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### 1.3. Summary of Clinical Pharmacology and Biopharmaceutics Findings:

A clinical need exists for an alternative to catheter-based, contrast X-ray angiography (XRA) for high resolution visualization of arterial disease. Catheter angiography requires an arterial puncture, uses potentially nephrotoxic X-ray dye, and exposes the patient to ionizing radiation. Magnetic resonance imaging (MRI) is non-invasive, and, in general, it provides excellent soft tissue delineation. Several techniques have been developed using MRI to image vascular structures. Current magnetic resonance angiography (MRA) techniques without the use of contrast agents (e.g., 2D time-of-flight MRA) are inadequate in imaging vascular disease.

Vasovist (MS-325), a new molecular entity, is a gadolinium-based contrast agent (a derivative of Gd-DTPA with lipophilic group appended to DTPA) developed for magnetic resonance angiography

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in adults with known vascular disease. MS-325 is the product development code for the drug product containing trisodium-[(2-(R)-[(4,4-diphenylcyclohexyl) phosphonooxymethyl]-diethylenetriamine pentaacetato) (aquo)gadolinium(III)] as the active substance. The generic name of MS-325 is gadofosveset. MS-325 is formulated at a concentration of 0.25 mmol/mL. The proposed clinical dose is 0.03 mmol/kg via IV injection.

The Human Pharmacokinetics and Pharmacodynamics section of the submission is based on the results of six clinical studies involving 228 subjects (197 treated with vasovist and 41 treated with placebo). A summary of different clinical studies conducted is given below:

Study		Conclusion/Findings
MS-325-01A	Double blind, placebo controlled, safety, tolerance and PK in healthy human volunteers at 0.01, 0.03, 0.05 mmol/kg of IV bolus injection	The increase in C <sub>max</sub> and AUC was not linear with the increase in dose
MS-325-01B	Open label, Phase I, safety, tolerance and PK study at 0.05 mmol/kg	MS-325 is safe and well tolerated
MS-325-01C	Rising single dose (0.01- 0.15 mmol/kg), safety, tolerance, and PK study as IV bolus or 5 minute infusion	In the high dose group and in placebo renal tubule cells were observed in urine sediments
MS-325-06	A phase II study to evaluate the safety in patients with arterial vascular occlusive disease and safety and PK in patients on warfarin therapy	Overall, there was no significant difference in PK parameters of MS-325 in patients on warfarin therapy and the control group.
MS-325-07	A phase II study to evaluate safety and PK of MS-325 in subjects with varying degree of renal insufficiency	The exposure (AUC) increased almost two fold in patients with moderate and severe renal impairment. The half-life increased from 19 hrs in normal subjects to 49 hours in patients with moderate renal impairment to 70 hours in patients with severe renal impairment.

MS-325-16	A phase II study to evaluate the safety and PK of 0.05 mmol/kg MS-325 in subjects with moderate hepatic impairment and normal hepatic function	The moderately hepatic-impairment group showed no clinically important differences in PK parameters compared with an age-matched group with normal hepatic function.
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Another study (MS-325-02) reviewed was a randomized, double-blind, phase II, multicenter, dose finding study. This study was conducted in patients with aortoiliac occlusive disease and in patients with peripheral vascular disease or aortic aneurism.

**Mechanism of Action:** MS-325 binds to plasma proteins reversibly. The plasma protein binding renders MS-325 a favorable attribute for imaging of the vascular system with magnetic resonance imaging. In vivo human and pre-clinical protein binding extends the residence time of the drug in vascular space, thereby extending the imaging period. The protein binding of MS-325 increases the relaxivity greater than five-fold in plasma solution (compared to that in aqueous solution).

The potency of a magnetic resonance imaging contrast agent is related to its magnetic efficiency, or relaxivity. At a given concentration, a high relaxivity contrast agent alters the water proton relaxation time (T1) more efficiently than low relaxivity agent. This results in a greater proton relaxation rate ( $1/T1$ , in units of  $\text{sec}^{-1}$ ) which is detected as a brighter MR image signal intensity. The relaxation rate, or  $1/T1$  is used in describing contrast agent effects because, unlike T1 relaxation times, relaxation rates can be added arithmetically to assess their cumulative effects. The observed relaxation rate, in general, is linearly dependent on the concentration of paramagnetic species (MS-325). The relaxivity, R1, is defined as the slope of this dependence, and is expressed in units  $\text{mM}^{-1}\text{s}^{-1}$ .

**Pharmacodynamics:** The pharmacodynamics of MS-325 was determined by measuring the ex vivo plasma  $1/T1$ . A single escalating, bolus, IV dose of MS-325 was initially given to healthy volunteers to elucidate PD behavior of the drug (CSR MS-325-01A, CSR MS-325-01B, and CSR MS-325-01C). The high relaxivity of MS-325 is attributed to its protein binding. With increasing dose,  $1/T1$  increased and remained elevated throughout the imaging window of one hour post-dose. This is shown in Figure 1. At 10 min post-dose in Study MS-325-01A, the blood  $1/T1$  values for doses of 0.01, 0.025, and 0.05 mmol/kg were found to be 5, 12, and 20/seconds, respectively. For the same doses at 1 hr the  $1/T1$  was 4, 9, and 14/sec, respectively. These results imply that with increasing dose there should be an increase in contrast images. This was however, not observed in clinical efficacy trials. It does not appear that there is a direct relationship between relaxation time and image contrast.

**Basic PK Parameters:** A study at 0.03 mmol/kg (Study MS-325-16) in healthy volunteers showed that the plasma concentration-time profile conforms to an open two-compartment model. MS-325 concentration declined rapidly during distribution phase, ( $t_{1/2\alpha}$ ) ( $0.48 \pm 1.1$  hours) and more slowly during disposition phase, ( $t_{1/2\beta}$ ) ( $16.3 \pm 2.6$  hours). The mean total clearance was  $6.6 \pm 1.0$  mL/h/kg (i.e. 7.7 mL/min for a 70 kg person). Total clearance, Cl(t), and renal clearance Cl(r), values increased with the dose of MS-325. Total clearance and renal clearance are similar since urinary excretion is the

main route of elimination of MS-325. The mean terminal plasma half-life of MS-325 did not vary remarkably with dose (range: 13.4 to 18.0 hrs). The steady-state volume of distribution,  $V(ss)$ , tended to increase slightly with an increase in dose above 0.05 mmol/kg. The  $V(ss)$  (0.016 L/kg at 0.03 mmol/kg) indicates that MS-325 is distributed to the extracellular space (CSR-MS-325-16).

In MS-325-1C single rising dose safety and PK study showed that at higher doses (0.125 and 0.15 mmol/kg) renal tubular cells were seen in urinary sediments. Renal tubular cells were also observed in urinary sediments in placebo group as well. The appearance of renal tubular cells and inclusion bodies in the urinary sediments is of concern. A few renal cells were also observed approximately **“24 hours” post-dose in one individual** (Subject No. 608) in dose group 0.100 mmol/kg. The sponsor failed to find out the reason behind these findings and simply attributed it to **“some other as yet unidentified study procedure.”** However, there were no clinically significant changes in any other parameters of renal functions. No renal cells were found in subjects receiving lower dose (0.05 and 0.075 mmol/kg) of MS-325 and proposed clinical dose of 0.03 mmol/kg. The details of this study have been communicated to Medical Officer Team Leader (Dr. Zili Li).

**Dose Finding Study:** In a dose finding study (Phase II (safety and clinical efficacy study) CSR-MS-325-02) three dose groups (0.01 mmol/kg, 0.03 mmol/kg, and 0.05 mmol/kg) in patients with carotid and peripheral arteries stenosis, no clear dose response was observed.

Primary efficacy endpoints were based on the 5( $\pm$ 4) minute scan for each side; left and right, for three arterial regions; iliac, femoral, and carotid.

1. The disease state was determined by the presence of clinically significant stenosis (>50% peripherals and >70% carotid) or non-clinically significant stenosis as determined by the blinded reader per body side, left and right, in all three arterial regions combined; iliac, femoral and carotid;
2. The location (i.e. zone) of clinically significant stenosis as determined by the blinded reader in all three arterial regions separately;
3. The location discrepancy blinded read used to determine the location of clinically significant stenosis in all three regions separately;
4. Simultaneous determination of disease and location of the most clinically significant stenosis in all three arterial regions separately;

For all regions combined (Table I), each 5( $\pm$ 4) minute post-MS-325 administration MRA image was analyzed in comparison to the X-ray angiography image (the gold standard) for sensitivity, specificity, accuracy and kappa for the determination of disease state of clinically significant stenosis in all three regions combined. **There was no clear dose response observed.**

Table I. Determining disease state for all three regions combined after 5(±4) minute post-MS-325 IV administration

Sensitivity %	88	86	89	64	100	76	73	80	79
Specificity %	100	67	75	100	85	88	82	75	79
Accuracy %	91	80	85	78	92	87	78	77	75
Kappa	0.79	0.52	NA	0.58	0.83	NA	0.57	0.55	NA

NA= not available

\*Comb = left and right sides were combined in a tertiary analysis in order to better summarize the data

Table II. Identifying the location of most clinically significant stenosis for all three regions for each side and combined at 5 min post MS-325 administration.

Accuracy %	100	0	67	100	60	71	67	100	75
Kappa	1.00	NA	NA	1.00	0.44	NA	0.54	1.00	NA
Accuracy %	100	50	75	100	100	100	67	100	83
Kappa	1.00	-0.33	NA	1.00	1.00	NA	0.40	NA	NA
Accuracy %	100	100	100	100	100	100	NA	100	100
Kappa	NA	NA	NA	NA	NA	NA	NA	NA	NA

\*Comb = left and right sides were combined in a tertiary analysis in order to better summarize the data

Table III. Simultaneous identification of disease state and location of stenosis for three regions after 5(±4) min post injection of MS-325

Accuracy %	67	0	40	73	67	71	70	89	75
Kappa	0.57	-0.29	NA	0.58	0.52	NA	0.61	0.80	NA
Accuracy %	100	50	75	100	100	100	67	100	83
Kappa	1.00	-0.33	NA	1.00	1.00	NA	0.40	NA	NA
Accuracy %	100	100	100	71	100	88	60	60	55
Kappa	1.00	1.00	NA	0.50	1.00	NA	-0.18	0.17	NA

\*Comb = left and right sides were combined in a tertiary analysis in order to better summarize the data

The analysis for identification of the location of the most clinically significant stenosis by arterial region was performed for each vascular region separately because each region was divided into a different number of zones due to anatomical differences in vessel anatomy. **The results showed (Table II) that in iliac region a good correlation between MRA and X-ray angiography images with a slight trend toward improvement with increasing dose. In the femoral artery also no dose response was observed.** In carotid region, results showed a good correlation between MRA and X-ray image, with 100% accuracy for all three doses 0.01, 0.03 and 0.05 mmol/kg, respectively.

Primary endpoints, the determination of disease state and the identification of the location of the most clinically significant stenosis were analyzed for each body region for accuracy and kappa at 5( $\pm$ 4) minute post-MS-325 administration time point. The accuracy for left iliac was 67%, 73%, 70% for 0.01 and 0.03 and 0.05 mmol/kg dose, respectively (Table III). **Therefore, no dose response was observed.** Similarly, for femoral (left) the accuracy was 100%, 100% and 67% for 0.01, 0.03, 0.05 mmol/kg dose, respectively. **Therefore, it is possible that the result of the images may be because of device itself. It may not have much to do with the drug itself.** For carotid artery % accuracy for combined (left and right side) was 100%, 88% and 55% for 0.01, 0.03 and 0.05 mmol/kg dose, respectively. **This actually shows a negative dose response.**

The pre-contrast MRA images were compared to the 5( $\pm$ 4) minute post-MS-325 administration MRA images for sensitivity, specificity, accuracy and kappa for determining disease states. **For all regions combined: the results showed upon comparison between pre- and post-contrast images, there was no clear dose response.** For iliac region the accuracy for three 0.01, 0.03, 0.05 mmol/kg doses for pre-contrast were 60%, 95% and 56%, respectively and 60%, 79%, and 90% for post-contrast images.

There is no overall dose response noted in phase II, dose selection clinical trials (MS-325-02). Therefore, the efficacy of this agent is at best questionable. Therefore, this reviewer recommends that the risk/benefit ratio should be evaluated with the integrated information from Phase III studies.

The sponsor claims that the dose selected for clinical studies (0.03 mmol/kg) was due to its optimal safety and efficacy for the following reasons:

- The proportion of uninterpretable scans at MS-325 0.01 mmol/kg was substantially greater than zero for each reader (4.7%, 12.5%, and 4.7%) and larger than that for 0.03 mmol/kg dose (0.0%, 0.0% and 1.3%, respectively).
- Specificity improved at the MS-325 0.03 mmol/kg dose compared to 0.01 mmol/kg for all three blinded readers (by 6.8%, 13.8%, and 6.0%)
- Sensitivity improved at 0.03 mmol/kg compared to 0.01 mmol/kg (14.7%, 13.2%, 9.7%) for all three blinded readers, and two out of three showed an increase in sensitivity improvement compared to pre-contrast at 0.03 mmol/kg compared to 0.01 mmol/kg.

**QTc Studies:** From ECG data it does not appear that Vasovist prolong QT or QTc interval. There was no dose dependent increase in QTc interval. There was no significant increase in mean QT or QTc value from the baseline at 45 min or 1 hr post-injection (Study MS-325-12). For example, the ECG results showed that **"twenty-two patients (8.4%) had a total of 25 increases in QTc interval that were >30 ms including 19 patients with increases that were 30-60 msec, and four patients had a single increase that was >60 msec. In addition there were 25 patients (9.5%) who had a total of 28 decreases, 30 msec. Of the increases, one patient had both 30-60 msec increase and a >60msec increase. Of the patients with >30 msec QTc changes, seven patients had QTc values that were in the**

normal range at Baseline and borderline or high at 45 minutes post-dosing. All of these returned to within the normal range by the 72 Hour post dosing time point. Three other patients had borderline QTc values at baseline which elevated to high at either 45 min post dose (one patient, whose QTc was in the normal range at the 72 Hour visit) or at the 72 Hour visit (two patients).”

From these results it appears that there may be QTc prolongation due to the drug administration, but the mean QTc values do not show an appreciable increase in QT or QTc. A label warning about Vasovist effect on QTc is warranted.

**Drug-Drug Interaction:** Warfarin may be used in subjects with vascular disease requiring diagnostic MR imaging, and because both warfarin and MS-325 bind to human serum albumin (HAS), a study was undertaken in subjects with arterial vascular disease to assess potential PK/PD interactions of MS-325 and warfarin. A Phase II study was conducted to evaluate the safety of MS-325 in patients with arterial vascular occlusive disease and the safety and pharmacokinetics of MS-325 in patients on warfarin therapy. The dose of MS-325 injected was 0.05 mmol/kg BW (0.2 mL/kg diluted to 25 mL with normal saline for injection, USP), administered as intravenous bolus at a rate of 1.5 mL/sec, followed by 30 mL normal saline flush. Normal saline for injection was used as a placebo. Variable individual doses of warfarin as prescribed by patient’s physicians were used (recorded dose range 2.5-12 mg 1-4 times a week). Effect of warfarin on PK of MS-325 was evaluated by comparing the PK parameters of MS-325 at specific time points post-dose in TEST (MS-325 plus warfarin) and REF (MS-325 alone) groups. The effect of warfarin on PD of MS-325 was evaluated by comparing change in plasma relaxation rate  $\Delta(1/T1)$ , at specific time points post-dose in TEST and REF cohorts of the PK group. Effect of MS on PD of warfarin was evaluated. The results showed that the PK profile of MS-325 was unaltered in subjects on concurrent warfarin therapy as compared to those who did not receive warfarin. The concentrations in plasma and PUF were nearly super-imposable in the presence or absence of warfarin.

**PK Parameters in Hepatic Impaired Patients:** Hepatic impaired (HI; Child-Pugh class B) and age-matched normal (AMN) liver function groups had similar group mean PK parameters, including no overall differences in urinary or total elimination of MS-325. Fecal elimination, while small in both groups, was reduced in the HI group relative to the AMN group. Average protein binding was not different between HI (69.4%) and AMN (70.2%) groups immediately following injection, and the amount of MS-325 bound to serum proteins as a function of concentration was similar between the 2 groups.

Despite similar results between group means, 1 HI subject (Patient 13), who had much lower plasma albumin than other patients in both the HI and AMN groups (<2.8 g/dL vs. 3.9-4.6 g/dL), exhibited notably faster plasma elimination relative to the other HI and AMN subjects. This behavior is consistent with the mechanism of action of MS-325 binding to albumin, and does not represent a likely efficacy concern for these patients.

**PK Parameters in Renal Impairment:** In renal impaired patients the renal clearance decreased substantially in patients with moderate and severe renal impairment. The exposure (AUC) increased almost two fold in patients with moderate and severe renal impairment. The half-life increased from 19 hrs in normal subjects to 49 hours in patients with moderate renal impairment to 70 hours in patients with severe renal impairment. This prolonged terminal half-life raises the possibility of in-vivo dissociation of Gd-fosveset. Therefore, this reviewer recommends that the in-vivo stability of Gd-fosveset be addressed by the sponsor before the drug can be approved. The potential in-vivo dissociation of Gd-fosveset can lead to free gadolinium ion toxicity and complexation of free ligand with calcium and other metals. A decrease in plasma calcium ion concentrations can lead to hypocalcemia. It is therefore recommended that the sponsor conduct a study determining the extent of in-vivo dissociation of Gd-fosveset in patients with renal insufficiency by comparing the amount of zinc-fosveset and calcium-fosveset in the urine collected in these patients with those in healthy volunteers.

#### SIGNATURES

Christy S. John, Ph.D.  
Clinical Pharmacology Reviewer  
Division of Pharmaceutical Evaluation II  
Office of Clinical Pharmacology and Biopharmaceutics

Date: December 1, 2004

Young Moon Choi, Ph.D.  
Team Leader  
Division of Pharmaceutical Evaluation II  
Office of Clinical Pharmacology and Biopharmaceutics

Date: December 1, 2004

#### **OCPB Briefing Meeting:**

**Date: August 16, 2004**  
**Time: 1:00 PM**  
**Location: 13B45**  
**Level: Optional inter-division**

**CC:**  
**NDA 21-711**  
**HFD-160**  
**DPE2 (Malinowski, Hunt, Choi, John)**

## 2. Question-Based Review:

### 2.1 General Attributes of the Drug

#### Q. What are the general attributes of Vasovist/gadofosveset?

A. Gadofosveset (also called MS-325 or Vasovist or Angiomark) is a white to slightly yellow powder as a drug substance and a clear, colorless to pale yellow solution as a drug product. The drug substance is a derivative of DTPA, a chelating agent with a diphenylcyclohexyl phosphonate group appended in the backbone. The chemical name of drug is trisodium-{(2-@-[(4,4-diphenylcyclohexyl)phosphonooxymethyl]-diethylenetriaminepentaacetato)(aquo) gadolinium (III)}. The chemical structure is shown in Figure I. It's molecular weight is 975.88. The drug product is extremely water soluble, with limited solubility in common organic solvent.

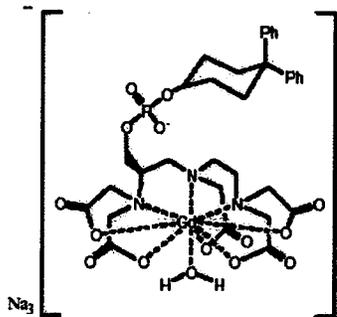


Figure I. Chemical Structure of Gadofosveset (Vasovist)

#### Q. How is the drug product formulated? Are there any issues with formulation?

A. The MS-325 drug product consists of a 0.250 mmol/mL (244 mg/mL) solution of drug substance (gadofosveset) with 0.1% (w/w fraction) of ligand excipient (fosveset). The

The pH of final product is adjusted to between 6.5 and 8.0. MS-325 is provided as a colorless to pale yellow sterile solution with an osmolality of approximately 825 mOsm/kg. It is supplied in 10 mL containing 10 and 20 mL vials containing 15 mL of MS-325 in a Type I glass vial. There are no issues with formulations.

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#### Q. What is the proposed mechanism of action of MS-325?

A. MS-325 binds to plasma proteins reversibly. The plasma protein binding renders MS-325 a favorable attribute for imaging of the vascular system with magnetic resonance imaging. In vivo human and pre-clinical protein binding extends the residence time of the drug in vascular space, thereby extending the imaging period. The protein binding of MS-325 increases the relaxivity greater than five-fold in plasma solution (compared to that in aqueous solution).

**Q. What is the proposed dosage and route of administration of MS-325?**

A. The proposed dosage of MS-325 is 0.03 mmol/kg to be administered by bolus intravenous injection.

**Q. What are other gadolinium contrast agents approved by the Agency?**

A. There are four gadolinium based contrast agents approved by the Agency. These include magnevist, prohance, omniscan, and optimark. The dose approved for all these agents is 0.1 mmol/kg.

**2.2 General Clinical Pharmacology:****Q. What are the basic pharmacokinetic properties of MS-325?**

A. Following a bolus injection with 0.03 mmol/kg MS-325, plasma concentrations of gadofosveset declined in a bi-exponential manner, i.e., more rapidly during the distribution phase than during the elimination phase, which begins at approximately two hours post-injection (Figure II). The mean plasma concentration at one hour post-injection (0.24 mmol/L) was 56% of the concentration recorded at 3-minute post-injection (0.43 mmol/L). The mean half-life of the distribution phase ( $t_{1/2\alpha}$ ) was 0.48 hours, the mean half-life of the elimination phase, ( $t_{1/2\beta}$ ), was 16.3 hours in healthy volunteers. At dose near the proposed clinical dose of 0.03 mmol/kg (0.01 to 0.05 mmol/kg), PK parameters were proportional to dose and plasma MS-325 were directly correlated to changes in  $1/T_1$ . At higher doses ( $>0.05$  mmol/kg) the AUC(0-inf) was not linearly correlated to the dose, due to reduced protein binding of the drug and more efficient clearance at higher plasma concentration.

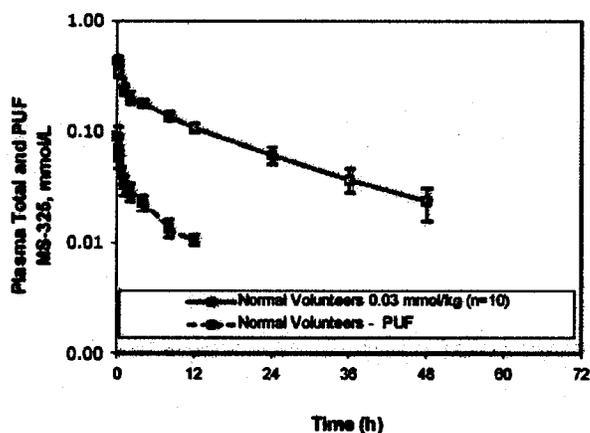


Figure II. Mean plasma (SD) and plasma ultra filtrate concentration of MS-325 with time in healthy volunteers

The PK profile of MS-325 has been studied over a wide range of doses (0.01 to 0.15

Table IV. PK parameters of MS-325 administered as IV infusion over different dose ranges (0.05 to 0.15 mmol/kg)

Dose Group	Dose Level (mmol/kg)	Length of Dose (seconds)	C <sub>max</sub> (µg/mL)	AUC (µg-hr/mL)	t <sub>1/2</sub> (α) (hr)	t <sub>1/2</sub> (λ) (hr)	CL (L/hr/kg)	V <sub>ss</sub> (L/kg)	CL <sub>R</sub> (L/hr/kg)
D	0.050	30	98.3	962	0.354	13.9	0.0083	0.160	0.0061
F	0.075	30	129	1327	0.693	15.0	0.0090	0.184	0.0074
H	0.100	30	151	1435	0.560	14.9	0.0111	0.227	0.0093
E	0.075	75	134	1227	0.397	13.4	0.0097	0.179	0.0073
G	0.100	75	162	1454	0.676	14.7	0.0109	0.218	0.0093
J-1	0.125	75	186	1730	0.567	14.6	0.0116	0.229	0.0099
I	0.150	75	219	1782	0.701	13.8	0.0135	0.249	0.0109

mmol/kg), or 0.3 to 5 times the suggested dose of 0.03 mmol/kg. In healthy volunteers, the mean urinary excretion of MS-325 ranged from approximately 71 to 82% over a period of 168 hours after a dose of 0.025 to 0.05 mmol/kg.

**Q. Are the pharmacokinetics of vasovist linear over varying dose ranges in healthy human volunteers?**

A. Single escalating bolus IV doses of MS-325 were initially given to healthy volunteers to examine PK profile of the drug (CSR MS-325-1A and CSR MS-325-01C) (Table IV). In study MS-325-01A, the drug was administered at doses of 0.01, 0.025, and 0.05 mmol/kg over 30 seconds. In study MS 325-01C, IV bolus doses of MS-325 were given over 30 seconds at doses of 0.05, 0.075, and 0.1 mmol/kg and over 75 seconds at doses of 0.075, 0.1, 0.125 and 0.15 mmol/kg. In the early PK studies (CSR MS 325-01A and CSR MS-325-01C) where doses between 0.01 and 0.15 mmol/kg were investigated, plasma MS-325 concentrations were found to increase with dose administered. However, the area under curve from zero to infinity increased less than proportionally in response to increase in dose. This trend is shown in Figure III. As the dose increased by 1 : 2.5 : 5 : 7.5 : 10 : 12.5 : 15, the AUC(0-inf) increased by ratios of 1 : 2.4 : 3.7 : 4.8 : 5.4 : 6.5 : 6.6. This observation is consistent with known protein binding characteristics of MS-325, in which the fraction bound decreases at plasma concentrations of MS-325 greater than 0.15 nM, resulting in a higher unbound fraction available for renal excretion.

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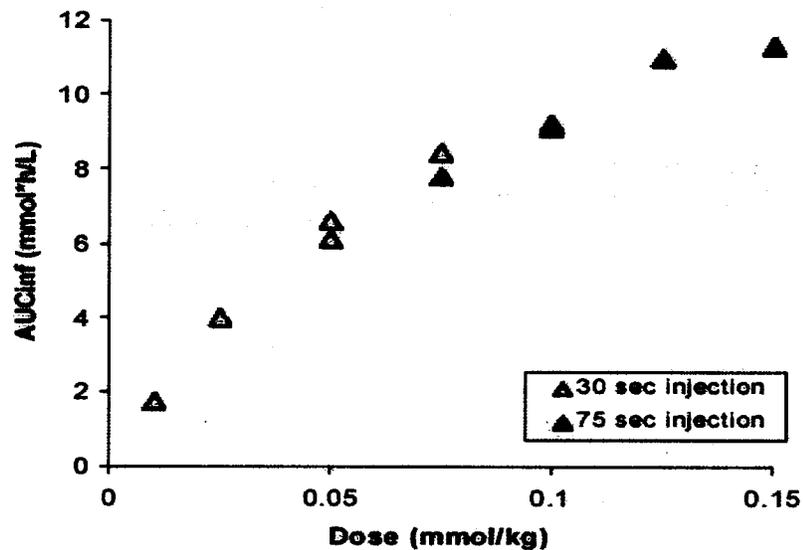


Figure III. The relationship between mean MS-325 AUC(0-inf) with varying dose of MS-325 in subjects receiving single IV dose

The relaxivity of MS-325 relies on its binding to plasma albumin, a decrease in the protein binding at higher doses results in a decrease in relaxivity of the agent. With increasing dose, the magnetic effect, or plasma  $\Delta 1/T1$ , also increase. However, increase in plasma  $\Delta 1/T1$  are not proportional to increasing dose. This non-proportionality of the parameters measured in this study is most likely explained by a decrease in percent of MS-325 bound to plasma protein with increasing dose.

**Q. What is the effect of age and gender on pharmacokinetics of MS-325?**

A. There is no difference in pharmacokinetics of MS-325 between male and female. There is also no significant difference in kinetics between adults and elderly 65 years and over. Adjustment of dose in geriatric population is not necessary. The PK parameters are shown in Table V.

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Table V. Pharmacokinetic parameters mean and (%CV) in sub-population of subjects at a dose of 0.05 mmol/kg

PARAMETER	Male 0.05 mmol/kg	Female 0.05 mmol/kg	Adult (< 65 years) 0.05 mmol/kg	Elderly (65 years and over) 0.05 mmol/kg
No. of Subjects	46	18	57	7
C <sub>max</sub> , mmol/L	0.64 (22)	0.69 (28)	0.67 (23)	0.54 (26)
AUC(0-inf), mmol*h/L	7.19 (16)	6.50 (15)	6.95 (16)	7.39 (14)
t(1/2term), h	19.6 (21)	17.3 (18)	18.7 (21)	21.1 (18)
V(ss), L/kg	0.169 (8)	0.161 (12)	0.165 (10)	0.176 (9)
Cl(t), mL/h/kg	7.12 (16)	7.84 (14)	7.37 (16)	6.89 (15)
Cl(r), mL/h/kg	5.55 (20)	6.07 (19)	5.72 (20)	5.49 (21)
X(u), %	76.1 (11)	76.5 (14)	76.0 (12)	77.7 (6)
X(f), %	5.45 (47)	6.12 (39)	5.67 (53)	5.48 (38)

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**Q. What are the characteristics of exposure-response relationship for efficacy?**

A. The sponsor evaluated three different doses (0.01 mmol/kg, 0.03 mmol/kg and 0.05 mmol/kg) for efficacy trials. Three different regions (carotid, femoral, and iliac) were analyzed for comparison to X-ray angiography (truth/gold standard) for sensitivity, specificity, and accuracy. All patients evaluated for efficacy received MS-325 and were scanned at 5(±4) minute post-MS-325 administration. The primary efficacy endpoints and brief analysis is discussed below:

**Primary Efficacy Endpoints and Analysis:**

Primary efficacy endpoints were based on the 5(±4) minute scan for each side; left and right, for three arterial regions; iliac, femoral, and carotid.

1. The disease state was determined by the presence of clinically significant stenosis (>50% peripherals and >70% carotid) or non-clinically significant stenosis as determined by the blinded reader per body side, left and right, in all three arterial regions combined; iliac, femoral and carotid (Figure IV);
2. The location (i.e. zone) of clinically significant stenosis as determined by the blinded reader in all three arterial regions separately;
3. The location discrepancy blinded read used to determine the location of clinically significant stenosis in all three regions separately;
4. Simultaneous determination of disease and location of the most clinically significant stenosis in all three arterial regions separately;

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Figure IV. Carotid zones, iliac zones, and femoral zones used to determine location of clinically significant stenosis

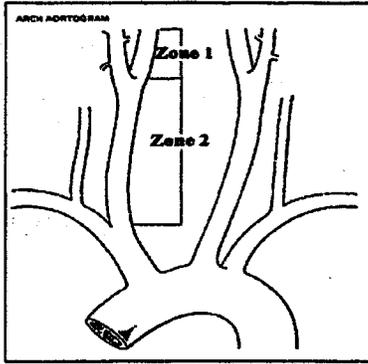


Figure 1. Carotid zones used to determine location of clinically significant stenosis.

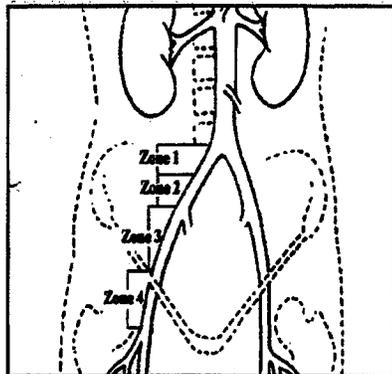


Figure 2. Iliac zones used to determine location of clinically significant stenosis.

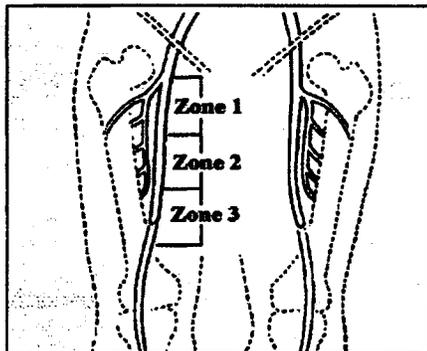


Figure 3. Femoral zones used to determine location of clinically significant stenosis.

The primary 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. The analysis was performed based on the data from the blinded readers in each Phase III study. Sensitivity was calculated as the number of correctly identified abnormal vessels divided by the total number of abnormal vessels. Specificity was calculated as the total number of correctly identified normal vessels divided by the total number of normal vessels. Accuracy was calculated as the number of correctly diagnosed vessels divided by the total number of vessels that were XRA interpretable, and it was used as the overall measure of sensitivity and specificity in an aggregate sense.

Efficacy end-points were based on data from both independent blinded readers (primary efficacy endpoint) at a central lab and institutional readers at each study center (secondary efficacy endpoint). For the blinded reads, three independent blinded readers each read and interpreted all MRA images. In addition, two independent blinded readers read and interpreted all XRA images for each patient enrolled. Institutional readers at each study center also read and interpreted **each patient's MRA and XRA image**. Each reader was to determine the percent stenosis of the most severely stenotic vessel on both the right and left sides (when available). The eligible vessels were the infra-renal abdominal aorta (IRAA), and the common iliac artery (CIA), external iliac artery (EIA), and common femoral artery (CFA) on each side. The standard of reference (SOR) for the primary analysis was generated by averaging the percentage of stenosis identified in a specific vessel segment/patient by each of the three blinded XRA readers. A >50% stenosis was considered to be a clinically significant disease. Each blinded reader evaluation of the MRA images was compared to the SOR for the presence or absence of clinically significant disease on individual vessel basis and diagnostic efficacy was assessed using receiver operating characteristic (ROC) analysis. The Area under the ROC Curve (AUC) for MS-325 contrast-enhanced MRA versus the non-contrast MRA was used as the primary variable to characterize the efficacy.

In addition, sensitivity, specificity and the proportion of uninterpretable scans for post-contrast MRA, pre-contrast MRA, and the difference between post- and pre-contrast MRA were tabulated as a function of dose. The primary efficacy analysis was based on the independent blinded reader assessments of the presence or absence of clinically significant stenosis (defined as >50% stenosis) in each vessel. The institutional reader assessment was performed as a secondary efficacy variable.

#### Determining Disease State in All Three Arterial (carotid, iliac and femoral) Regions Combined

All regions Combined:

Each 5(±4) minute post-MS-325 administration MRA image was analyzed in comparison to X-ray angiography image (the gold standard) for sensitivity, specificity, accuracy and kappa for the determination of disease state of the clinically significant stenosis in all three regions combined. The sponsor claims that MS-325 enhanced MRA performed well

compared to conventional X-ray angiography. However, no clear dose response was seen (Table VI).

**Table VI. Determining Disease State for All Three Regions Combined at the 5(±4) Minute Post MS-325 Administration**

<b>Sensitivity %</b>	88	86	89	64	100	76	75	80	79
<b>Specificity %</b>	100	67	75	100	85	88	82	75	79
<b>Accuracy %</b>	91	80	85	78	92	87	78	77	75
<b>Kappa</b>	0.79	0.52	NA	0.58	0.83	NA	0.57	0.55	NA

NA= not available

\*Comb = left and right sides were combined in a tertiary analysis in order to better summarize the data

Identification of the location of most clinically significant stenosis by arterial region:

If the stenosis was determined to be clinically significant (>50% stenosis in the iliac and femoral regions or >70% stenosis in the carotid region) then the MRA and X-ray blinded readers identified the location of stenosis. The analysis was performed for each vascular region (carotid, iliac and femoral) separately because each region was divided into different number of zones due to anatomical differences in vessel anatomy. Each 5(±4) minute post-MS-325 administration MRA scan was analyzed for accuracy and kappa for this endpoint.

**Identifying the Location of the Most Clinically Significant Stenosis for all Three Region at 5 min Post-MS-325 Administration**

**Iliac Region:** In the iliac region, results showed good correlation between MRA and X-ray angiography images. There was no dose response observed.

**Femoral Region:** In the femoral region, results showed good correlation between MRA and XRA at all doses. No dose response was observed

**Carotid Region:** In the carotid region, results showed excellent correlation between the MRA and X-ray image, with 100% accuracy at all doses (No dose response).

**Simultaneous Identification of Disease State and Location by Arterial Region:**

**Iliac Region:** Results showed good correlation with X-ray angiography for the iliac region. Accuracy results showed a clear trend toward better performance with increasing dose. Accuracy for the right iliac was 67% and 89% for the 0.03 and 0.05-mmol/kg dose groups, respectively, while 0% for the 0.01 mmol/kg dose group. Accuracy for the left iliac artery was 73% and 70% for the 0.03 and 0.05 mmol/kg dose groups, respectively, while 67% for the 0.01-mmol/kg dose group (Table VII).

Femoral Region: In the femoral region, MRA performed well compared to X-ray angiography, but there was no improvement with increasing dose.

Carotid Region: In the carotid region, MRA performed well compared to XRA images, but there was no improvement with increasing dose.

The Simultaneous Identification of Disease State and Location of Stenosis for All Three Regions.

Table VII. Dose response (simultaneous identification of disease and location of stenosis) of MS-325

Accuracy %	67	0	40	73	67	71	70	89	75
Kappa	0.57	-0.29	NA	0.58	0.52	NA	0.61	0.80	NA
Accuracy %	100	50	75	100	100	100	67	100	83
Kappa	1.00	-0.33	NA	1.00	-1.00	NA	0.40	NA	NA
Accuracy %	100	100	100	71	100	88	60	60	55
Kappa	1.00	1.00	NA	0.50	1.00	NA	-0.18	0.17	NA

\*Comb = left and right sides were combined in a tertiary analysis in order to better summarize the data

Secondary Efficacy Analysis:

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**Determining Disease State at the 5(±4) minute post-MS-325 administration Timepoint by Arterial Region:**

Results for Iliac Region:

The results for determination of disease state at the 5(±4) minute post-MS-325 administration timepoint showed good correlation with X-ray angiography. A clear dose response was noted with best overall performance of MS-325.

Results for Femoral Region:

The sponsor claims that in the femoral region, results from this endpoint showed excellent correlation with X-ray angiography. No dose response was noted, sensitivity, accuracy, and specificity were at 100% at all dose levels.

Results for Carotid Region:

The sponsor claims good agreement with X-ray angiography. A negative dose response was noted; specificity, sensitivity and accuracy decreased with increasing dose.

**Pre-Contrast: Determining Disease State**

The pre-contrast MR images were compared to the 5(±4) minute post-MS-325 administration MRA images for sensitivity, specificity, accuracy for determining disease state.

For All Regions Combined: Pre-contrast and the 5(±4) minute post-MS-325 administration MRA scans were compared for determining the disease state in all three regions combined (Table VIII). It does not appear that there is a direct dose-response relationship between the dose and the quality of the images.

Table VIII. Comparison of Pre- and 5(±4) Minute Post-MS-325 Administration MRA Images for Determining Disease State for All Three Regions Combined

Sensitivity %	63	83	67	77	89	84	90	67	81
Specificity %	100	80	80	100	100	100	80	67	70
Accuracy %	73	82	75	85	95	91	85	67	77
Kappa	0.48	0.63	NA	0.70	0.90	NA	0.70	0.31	NA
Sensitivity %	88	86	89	64	100	76	75	80	79
Specificity %	100	67	75	100	85	88	82	75	79
Accuracy %	91	80	85	78	92	87	78	77	75
Kappa	0.79	0.52	NA	0.58	0.83	NA	0.57	0.55	NA

\*Comb = left and right sides were combined in a tertiary analysis in order to better summarize the data

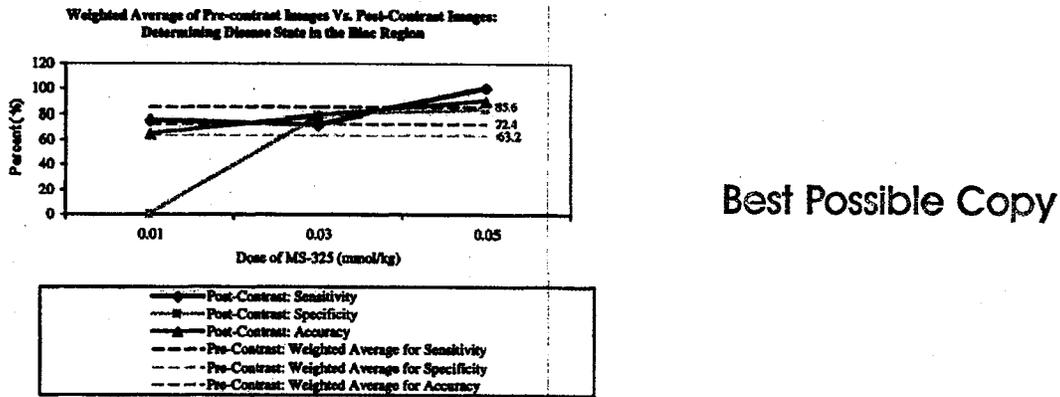


Figure V. Weighted average of pre-contrast images vs post-contrast images in the iliac region

**Iliac Region:**

In the iliac region, results showed that pre-contrast images performed poorly compared to X-ray angiography at 0.01 and 0.05 mmol/kg doses while performing well at the 0.03 mmol/kg dose (figure V). The weighted average for accuracy of all the pre-contrast images was 44.4%. When the 5(±4) minute post-MS-325 administration MRA image results across doses were compared to the weighted average of the pre-contrast images, results showed that the post contrast images performed better than pre-contrast images at

both the 0.03 and 0.05 mmol/kg (50% and 65%, respectively). The weighted average of pre-contrast vs post-contrast in determining the disease state degree of stenosis in the iliac region is shown in Figure V and Figure VI.

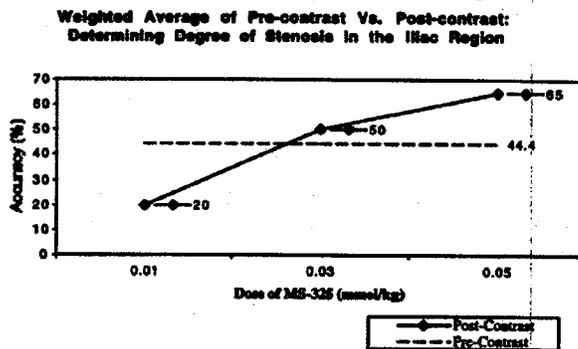


Figure VI. Determining degree of stenosis with pre-contrast and 5( $\pm$ 4) min post MS-325 administration for both sides (left and right) in the iliac region.

**Q. What are the design features of the clinical pharmacology and clinical studies to support dosing claim?**

A. The sponsor claims that optimal combination of efficacy and safety was obtained at the 0.03 mmol/kg dose due to the following reasons:

- The proportion of uninterpretable scans at MS-325 0.01 mmol/kg was substantially greater than zero for each reader (4.7%, 12.5%, and 4.7%) and larger than that for 0.03 mmol/kg dose (0.0%, 0.0% and 1.3%, respectively).
- Specificity improved at the MS-325 0.03 mmol/kg dose compared to 0.01 mmol/kg for all three blinded readers (by 6.8%, 13.8%, and 6.0%)
- Sensitivity improved at 0.03 mmol/kg compared to 0.01 mmol/kg (14.7%, 13.2%, 9.7%) for all three blinded readers, and two out of three showed an increase in sensitivity improvement compared to pre-contrast at 0.03 mmol/kg compared to 0.01 mmol/kg.
- The MS-325 0.05 mmol/kg dose was not substantially superior to the 0.03 mmol/kg in AUC improvement, nor did the uninterpretable rate improve at this higher dose. Thus, the higher 0.05 mmol/kg dose is not clinically justified for this study population.

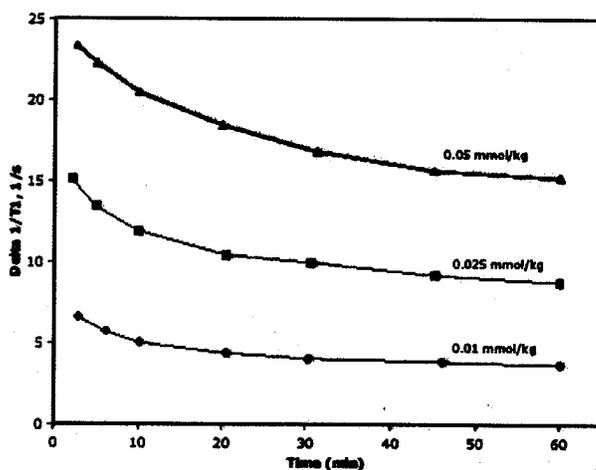
**Q. What is the basis for selecting the response endpoints and determining the efficacy of MS-325?**

The response endpoints were based upon % specificity, sensitivity and accuracy in three different arterial regions and determining the disease state and location of stenosis.

**Q. What is the relationship between in-vitro relaxivity and in-vivo efficacy of different doses MS-325?**

A. The potency of a magnetic resonance imaging contrast agent is related to its magnetic efficiency, or relaxivity. At a given concentration, a high relaxivity contrast agent alters the water proton relaxation time (T1) more efficiently than low relaxivity agent. This results in a greater proton relaxation rate ( $1/T1$ , in units of  $\text{sec}^{-1}$ ) which is detected as a brighter MR image signal intensity. The relaxation rate, or  $1/T1$  is used in describing contrast agent effects because, unlike T1 relaxation times, relaxation rates can be added arithmetically to assess their cumulative effects. The observed relaxation rate, in general, is linearly dependent on the concentration of paramagnetic species (MS-325). The relaxivity, R1, is defined as the slope of this dependence, and is expressed in units  $\text{mM}^{-1}\text{s}^{-1}$ . The in-vitro relaxivity of MS-325 at low concentrations (0.10 mM) in human plasma is 8-10 time that of commercially available MRI agent such as Magnevist (MS-325 IND 51,172;  $R1=53.5 \text{ mM}^{-1}\text{s}^{-1}$  as compared to  $5.0 \text{ mM}^{-1}\text{s}^{-1}$  GdDTPA, both measured at 20 MHz and 37 C). GdDTPA is the active ingredient in the commercial contrast agent Magnevist. GdDTPA does not bind to serum proteins.

The pharmacodynamics of MS-325 was determined by measuring the ex vivo plasma  $1/T1$ . A single escalating, bolus, IV dose of MS-325 was initially given to healthy volunteers to elucidate PD behavior of the drug (CSR MS-325-01A, CSR MS-325-01B, and CSR MS-325-01C). The results of these studies indicate that MS-325 is a potent MRI contrast agent. The high relaxivity of MS-325 is attributed to its protein binding. With increasing dose,  $1/T1$  increased and remained elevated throughout the imaging window of one hour post-dose. This is shown in Figure VII. At 10 min post-dose in Study MS-325-01A, the blood  $1/T1$  values for doses of 0.01, 0.025, and 0.05 mmol/kg were found to be 5, 12, and 20/seconds, respectively. For the same doses at 1 hr the  $1/T1$  was 4, 9, and 14/second, respectively.



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Figure VII. Change in relaxation time for different concentration of MS-325

In another study, in which MS-325 was given as an IV bolus dose of 0.05 mmol/kg (CSR MS-325-01B), early (approximate five minutes) arterial phase imaging demonstrated excellent and selective enhancement of non-coronary arteries. In addition all post-dose images were qualitatively graded as diagnostic, and an increase in image quality was always noted in going from pre-MS-325 to post-MS-325 images. High spatial resolution allowed visualization of tertiary branches that were not seen in the pre-contrast images. Quantitatively, the mean early arterial signal-to-noise ratio (SNR) and contrast-to-noise ratio (CNR) showed a decrease of only 11 to 15% on the delayed (60 minutes) MR images. The extended duration of the contrast effect for MS-325 is attributable to both its slower elimination from human blood, as well as its higher relaxivity in blood, when compared to other commercially available Gd contrast agents.

In study MS-325-06, the relationship between pharmacokinetics and pharmacodynamics was further explored. It was shown that  $\Delta 1/T1$  increases with increasing plasma MS-325 concentration. The relationship between the concentration of bound MS-325 and  $\Delta 1/T1$  is linear across a wide range of MS-325 plasma concentrations, demonstrating that protein-bound MS-325 is responsible for the high relaxivity observed for MS-325 in plasma. The non-linear relationship between total MS-325 concentration and  $\Delta 1/T1$  is due to the increasing proportion of unbound MS-325 (which has 5- to 8- fold lower relaxivity than the bound form) at higher concentrations (Figure VIII).

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Figure VIII. Plasma MS-325 concentration versus the change in relaxivity following MS-325 administration in subjects with vascular disease: Relationship of  $\Delta 1/T1$  to bound MS-325 concentration (left panel) and total MS-325 concentration (right panel)

The change in relaxivity observed in subjects with vascular disease in PK arm (n=14) at three time points (1, 5, and 30 minutes post-dose) in Study MS-325-06 was compared to the results of in vitro studies using human plasma. The change in relaxivity, measured in subjects with vascular disease (MS-325-06, subjects receiving only MS-325, n=7), was compared to a curve fit from a previous study (PTR-2003-37) over a corresponding plasma range. No differences were noted in vitro and in-vivo plasma binding.

**Q. How do the pharmacokinetic profiles of MS-325 in patients with vascular disease compare with healthy normal volunteers?**

A. There are no major differences in pharmacokinetic profiles of MS-325 in patients with vascular disease as compared to normal volunteers as shown in Table IX.

Table IX. PK parameters of MS-325 in healthy volunteers and vascular disease subjects

PARAMETER	Vascular Disease Subjects <sup>1</sup>	Healthy Volunteers <sup>2</sup>
	0.05 mmol/kg	0.05 mmol/kg
C <sub>max</sub> , mmol/L	0.56 (19)	0.70 (11)
AUC(0-inf), mmol*h/L	7.42 (23)	6.85 (14)
t(1/2term), h	22.2 (27)	18.8 (17)
V(ss), L/kg	0.17 (9)	0.16 (9)
Cl(t), mL/h/kg	7.07 (23)	7.42 (13)
Cl(r), mL/h/kg	5.22 (24)	6.23 (16)
X(u), %	73.1 (11)	83.6 (9)
X(f), %	5.49 (28)	4.81 (65)

1 Data from Study MS-325-06

2 Data from Study MS-326-16

**Q. Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure-response relationship?**

A. There is no active moiety identified or measured to assess pharmacokinetic parameters and exposure-response relationship. The drug product (gadofosveset) does not undergo any metabolism. Gadofosveset is the active moiety responsible for the action of the drug.

To assess the potential for metabolism of MS-325, a number of clinical, animal, and in vitro studies have been performed. These studies include analysis of unchanged drug (gadofosveset) and Gd during clinical studies, special analytical studies involving human urine pools, and non-clinical studies involving primates and rats, as well as a human liver microsome incubation study.

A number of human clinical studies have been performed in which total Gd and MS-325 (intact drug gadofosveset) were measured in plasma and urine. During the course of clinical development, plasma and urine samples were assayed for Gd and MS-325 (parent drug) over a range of concentrations representing different time periods post-dose. To investigate whether the drug remains intact in the body, the amounts of MS-325 and Gd in plasma and urine samples were compared. If present, metabolism would be expected to result in lower concentrations of MS-325 than Gd. The data showed that MS-325 and Gd concentrations correspond very well in plasma and urine in healthy subjects, vascular disease subjects, and subjects with varying degrees of renal impairment. The plots of log MS-325 concentration was plotted against log Gd concentrations. The slopes and

intercepts of all the graphs were examined and were close to 1 (range 0.98 to 1.07) and 0 (range **-0.0278 to 0.0421**) respectively, indicating no significant deviation from a linear relationship with a slope of unity.

Additional assays were performed on pooled urine samples collected from subjects participating in study MS-325-06 up to five days post-dose. The studies involved a number of advanced analytical techniques such as high performance liquid chromatography (HPLC-MS) and high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS), providing high sensitivity and specificity. The HPLC-ICP-MS analysis found no evidence of other Gd-containing species in either urine or plasma.

However, it was shown in study MS-325-16 that zinc excretion in urine increased from 556  $\mu\text{g}/24\text{hr}$  (baseline) to 837  $\mu\text{g}/24\text{hr}$  in normal volunteers group when a dose of 0.03 mmol/kg was administered. The increase in zinc concentration in age matched normal (AMN) hepatic function group was 1302  $\mu\text{g}/24\text{hr}$  (Table XX). This raises the possibility that Gd-fosveset may be undergoing in-vivo dissociation and trans-chelating with zinc and other elements such as magnesium and calcium.

**Q. Are there any cytochrome P450 enzymes involved in metabolism of MS-325?**

MS-325 was incubated with human microsomes at various concentrations. This study showed that MS-325 levels were unchanged, which implies that cytochrome P450 enzymes are not involved in the disposition of MS-325.

**Q. What is the major elimination pathway for MS-325?**

A. Renal excretion is the principle route of elimination for MS-325. In healthy subjects following an IV dose of 0.03 mmol/kg (CSR MS-325-16), the mean percent dose excreted in urine was 83.7% (range 79.0 to 94.0%). A small proportion of the dose (4.7%; range 1.1 to 9.3%) was eliminated in the feces. This indicates a minor role of biliary excretion.

**Q. Does vasovist prolong the QT or QTc interval?**

From ECG data it does not appear that Vasovist prolong QT or QTc interval. There was no dose dependent increase in QTc interval. There was no significant increase in mean QT or QTc value from the baseline at 45 min or 1 hr post-injection. However, **QTc studies were not conducted according to the Agency's concept paper.**

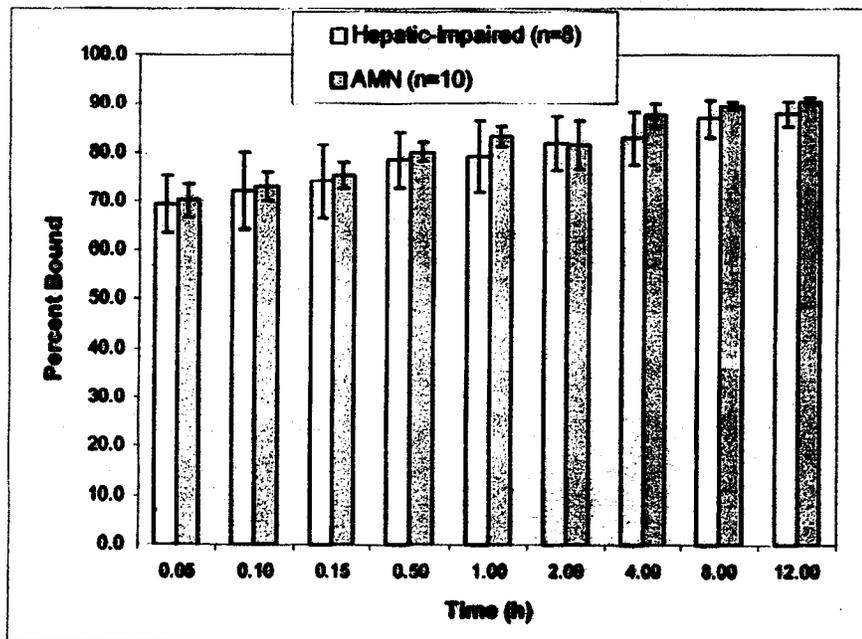
**2.3 Intrinsic Factors:**

**Q. How does the pharmacokinetic of vasovist compare in hepatic impaired (HI) patients with age matched normal (AMN) human volunteers?**

A. Overall, there were no important differences in the concentration-time profile of MS-325 or differences in the plasma protein bound fraction with time in the HI and AMN groups. There was no difference in the urinary elimination of MS-325, and >90% of the urinary elimination occurred in the first 2 hours. The small amount of fecal elimination seen in both groups demonstrates it is a minor pathway of elimination in both groups. On average,  $82.5 \pm 7.6\%$  and  $83.6 \pm 7.3\%$  of the administered dose was recovered in urine from the HI and AMN groups, indicating that MS-325 is excreted primarily via the kidneys. There was no clinically or statistically significant difference in renal elimination between the hepatic impaired and age-matched normals. Fecal elimination was slightly less in the HI group at  $2.7 \pm 1.9\%$  versus  $4.8 \pm 3.1\%$  for the AMN group, though this difference was not statistically significantly ( $p=0.08$ ).

**Q. What is the effect of hepatic impairment on plasma protein binding of MS-325?**

A. Hepatic impairment had no remarkable effect on the binding of MS-325 to plasma proteins. As shown in figure IX, the percent of total circulating MS-325 that was bound to plasma proteins was not substantially altered in subjects with hepatic impairment. The mean percent binding of MS-325 to plasma protein in hepatic impaired and aged-matched control subjects receiving a single IV dose of MS-325 (0.05 mmol/kg) is shown below:



Source: CSR MS-325-16, Section 11.3.4, Figure 11-2

Figure IX. Plasma binding of MS-325 in hepatic impaired patients and age matched normal volunteers with respect to time

Both groups showed a similar pattern of increased percent bound MS-325 as a function of time.

**Q. What is the impact of renal impairment on the clearance of MS-325?**

A. MS-325 is cleared through the kidneys, the pharmacokinetics of MS-325 were noticeably affected by renal impairment following a single 0.05 mmol/kg dose. MS-325 plasma concentrations were studied following a single IV dose of MS-325 (0.05 mmol/kg) in subjects with varying degrees (mild, moderate and severe) of renal impairment. Subjects were classified based upon creatinine clearance calculated using the Cockcroft-Gault (C-G) equation from baseline serum creatinine values. As renal function decreased, plasma concentration at a given time post-dose increased while the systemic clearance of MS-325 decreased. The renal clearance decreased substantially in patients with moderate and severe renal impairment. The exposure (AUC) increased almost two fold in patients with moderate and severe renal impairment. The half-life increased from 19 hrs in normal subjects to 49 hours in patients with moderate renal impairment to 70 hours in patients with severe renal impairment.

A prolonged half-life in renal insufficient patients can potentially cause in-vivo dissociation of Gd-fosveset that can potentially lead to free gadolinium ion toxicity and complexation of free ligand with calcium and other metals such as iron, magnesium etc. A decrease in plasma calcium ion concentrations can lead to hypocalcemia. It is therefore recommended that the sponsor conduct a study determining the extent of in-vivo dissociation of Gd-fosveset in patients with renal insufficiency. The sponsor should conduct a study whereby the amount of zinc-fosvest and calcium-fosveset be determined in urine at 1, 2, 4, 6, 8, 24, 48, 72 hr and up to 7 days in patients with renal insufficiency and compare the data to normal volunteers.

**2.4 Extrinsic Factors:****Q. What is the effect of warfarin on PK parameters of MS-325?**

A. A Phase II study was conducted to evaluate the safety of MS-325 in patients with arterial vascular occlusive disease and the safety and pharmacokinetics of MS-325 in patients on warfarin therapy. This study was a single-site, two-arm, single-dose study with open-label pharmacokinetic (PK) arm with two cohorts, PK Reference (REF; receiving study drug alone; 10 patients) and PK Test (TEST; receiving study drug with concomitant warfarin therapy; 10 patients) and a double blind randomized, placebo-controlled Safety Arm with a Safety Treatment cohort (Drug; 10 patients) and a Safety Placebo (Placebo; 10 patients) cohort. The dose of MS-325 injected was 0.05 mmol/kg BW (0.2 mL/kg diluted to 25 mL with normal saline for injection, USP), administered as intravenous bolus at a rate of 1.5 mL/sec, followed by 30 mL normal saline flush. Normal saline for injection was used as a placebo. Variable individual doses of warfarin as **prescribed by patient's physicians were used** (recorded dose range 2.5-12 mg 1-4 times a week). Effect of warfarin on PK of MS-325 was evaluated by comparing the PK parameters of MS-325 at specific time points post-dose in TEST (MS-325 plus warfarin) and REF (MS-325 alone) groups. The effect of warfarin on PD of MS-325 was evaluated

by comparing change in plasma relaxation rate  $\Delta(1/T1)$ , at specific time points post-dose in TEST and REF cohorts of the PK group. Effect of MS-325 on PK of warfarin was evaluated by comparing the concentrations of R- and S-forms of warfarin in the pre- and post-dose MS-325 plasma samples from the TEST cohort of the PK arm. Effect of MS on PD of warfarin was evaluated by comparing anticoagulant effect of warfarin in plasma samples obtained pre- and post-MS-325 dose in TEST cohort of PK arm.

PK parameters for MS-325 were derived from plasma concentration-time profiles obtained by the two assay methods, i.e. ICP-AES method for Gd and HPLC/UV method for MS-325. The overall urinary excretion (cumulative 0-168 h) determined using the two assay methods were similar. Gadolinium plasma levels were nearly equivalent to that of MS-325 on a molar basis. Approximately 75 to 80% of administered dose was recovered in urine, indicating that MS-325 is excreted primarily via the kidneys.

The PK variables derived from assay results obtained using ICP-AES method. The ratio of means along with the mean AUC and Cmax values, expressed on a molar basis, were similar between the TEST and the REF groups. The TEST/REF ratio is expressed as a percent. The TEST/REF mean ratios were contained between 90% confidence intervals. The PK variables in the TEST and REF groups were similar as indicated by the ratio and the confidence intervals. Thus, warfarin does not effect the pharmacokinetic parameters of MS-325. The plasma binding of MS-325 is also not changed when warfarin is co-administered.

## 2.5 General Biopharmaceutics

Not applicable

## 2.6 Analytical Section

**Q. How are the active moieties identified and measured in the plasma in clinical pharmacology and biopharmaceutics studies?**

A. [redacted] developed and validated the majority of methods used to support the PK studies. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) methods were developed to measure Gd in human plasma, PUF, urine, and feces. Methods using HPLC with UV detection were developed to measure MS-325 in plasma and urine, but due to the inability to reliably recover MS-325 in feces, it was not possible to develop a method for MS-325 in this matrix. All methods at [redacted] were performed in accordance with Good Laboratory Practices.

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For several PK studies, both Gd and MS-325 were measured in plasma and urine samples to assess whether there was any evidence for metabolism. If there were evidence of metabolism of MS-325, less MS-325 than Gd on a molar basis would be found (Report AR009). In addition a detailed analytical study was conducted involving pooled urine samples from the MS325-06 study (Report AR-003). Data from this study show that the HPLC-UV method used for the PK studies compares favorably to potentially more

specific methods such as HPLC with MS/MS detection, and HPLC with ICP-MS detection.

**Q. What are the lower and upper limits of quantification?**

A. ICP with MS detection was used at EPIX Medical, Inc. to measure levels of Gd in urine below the lower limit of quantitation (1 µg Gd/mL) of the ~~ICP-AES~~ ICP-AES method. This more sensitive method was employed to measure Gd levels in urine in late time points in Study MS-325-16 and in dialysate samples from Study MS-325-18. Methods Used for Measuring Gadolinium in Human PK Studies and Limits of Quantification

Matrix	Technique	Laboratory	Reference	Limit of Quantitation
Plasma, PUF, Urine, Feces	ICP-AES	<del>ICP-AES</del>	6754-104	1 µg Gd/mL (6.4 nmol/L)
Urine, dialysate	ICP-MS/MS	EPIX	EP-ICP-100	0.004 µg Gd/mL (0.025 nmol/L)

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### **3. Detailed Labeling Recommendations**

**At the present time label is not yet reviewed.**

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**4. APPENDICES.**

**4.1 Proposed Package Insert (Original and Annotated)**

**Package insert has not been reviewed so far.**

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       Trade Secret / Confidential (b4)

✓ Draft Labeling (b4)

       Draft Labeling (b5)

       Deliberative Process (b5)

#### 4.2 Individual Study Review:

MS-325-16 A Phase II study to evaluate the safety and PK of 0.05 mmol/kg MS-325 in subjects with moderate hepatic impairment

Patients with confirmed moderate hepatic impairment were enrolled based on FDA Guidance criteria. Hepatic-impaired patients, classified by Child-Pugh scores for moderate hepatic impairment (Class B), were screened and entered into the study under additional specified inclusion/exclusion criteria. A group of normal healthy (normal hepatic function) volunteers were age-matched to the HI group and entered under the same inclusion/exclusion criteria. Normal healthy volunteers were enrolled to measure the pharmacokinetics of MS-325 at the 0.03 mol/kg dose used in the Phase III studies.

Overall, there were no important differences in the concentration-time profile of MS-325 or differences in the plasma protein bound fraction with time in the HI and AMN groups. There was no difference in the urinary elimination of MS-325, and >90% of the urinary elimination occurred in the first 72 hours. The small amount of fecal elimination seen in both groups demonstrates it is a minor pathway of elimination in both groups. On average,  $82.5 \pm 7.6\%$  and  $83.6 \pm 7.3\%$  of the administered dose was recovered in urine from the HI and AMN groups, indicating that MS-325 is excreted primarily via the kidneys. There was no clinically or statistically significant difference in renal elimination between the hepatic impaired and age-matched normals. Fecal elimination was slightly less in the HI group at  $2.7 \pm 1.9\%$  versus  $4.8 \pm 3.1\%$  for the AMN group, though this difference was not statistically significantly ( $p=0.08$ ).

Dose in the HI and AMN groups: 0.05 mmol/kg body weight (0.2 mL/kg) as intravenous bolus injection at a rate of 1.5 mL/sec, followed by 30 mL normal saline flush. Dose in the NV Group: 0.03 mmol/kg body weight (0.12 mL/kg) as intravenous bolus injection at a rate of 1.5 mL/sec, followed by 30 mL normal saline flush.

The effect of moderate hepatic impairment on the pharmacokinetics of MS-325 was evaluated by comparing the following pharmacokinetic (PK) parameters for MS-325 between the HI and AMN subjects: AUC(0-last), AUC(0-inf), C<sub>max</sub>, t(1/2<sub>alpha</sub>), t(1/2<sub>beta</sub>), t(1/2<sub>term</sub>), mean residence time (MRT), total clearance (Cl(t)), renal clearance (Cl(r)), volume at steady state (V(ss)), % urine excretion (X(u)), % fecal excretion (X(f)), % total excretion (X(t)), and the unbound fraction of MS-325 at specific time points post-dose. The same parameters were determined for the NV group, which was dosed at 0.03 mmol/kg.

#### Procedures:

Pharmacokinetics of MS-325: Nineteen (19) serial plasma samples were obtained from all patients from Baseline through 14 days post-MS-325 injection, as well as urine (13 collection intervals) and feces samples. The time points for blood, urinary and fecal collections to assess the PK

parameters are shown below:

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Parameter	Prior to MS-325	D O S I N G	Minutes Post-MS-325				Hours Post-MS-325										Days Post-MS-325		
	Within 24 Hours		3	6	12	30	1	2	4	8	12	24 (±2)	36 (±2)	48 (±2)	72 (±2)	120 (±2)	144 (±2)	168 (±2)	10 & 14 <sup>a</sup>
Pharmacokinetic blood sampling <sup>2</sup>	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Pharmacokinetic urine sampling <sup>3</sup>																			
Pharmacokinetic stool sampling <sup>4</sup>																			

Plasma, urine and feces were assayed for gadolinium (Gd) using an ICP-AES assay, and urine samples found to have concentrations less than the limit of detection for the ICP-AES method were re-assayed using a more sensitive assay (ICP-MS) to determine final urine excretion. Plasma concentration of unbound MS-325, i.e. concentrations in plasma ultrafiltrate (PUF), was also determined using the ICP-AES assay. The concentration-time data were analyzed for PK parameters using compartment model-dependent (open, 2-compartment model) and non-compartmental methods employing the pharmacokinetic analysis software WinNonlin.

The following parameters were calculated: AUC(0-last), AUC(0-inf), Cmax, Tmax, t(1/2alpha), t(1/2beta), MRT, V(ss), Cl(t), Cl(r). Cumulative urinary (X(u)), fecal (X(f)), and total (X(t)) excretion was expressed as a percent of the administered dose. PK parameters of MS-325 in hepatic impaired and age-matched normals are shown in Table X. Plasma clearance of MS-325 is shown in Figure X.

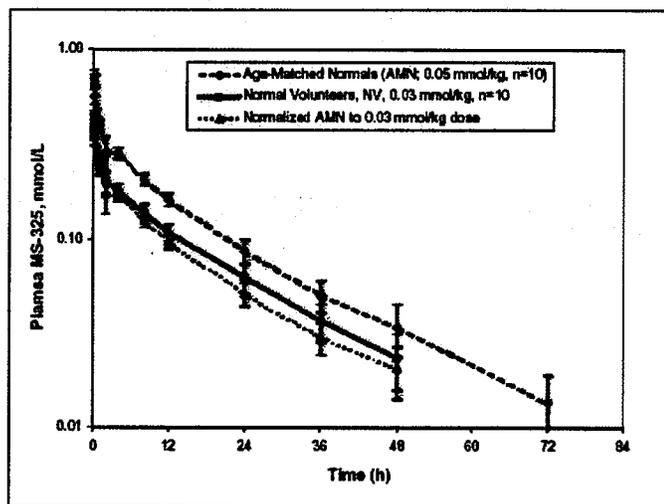
Table X. PK parameters of MS-325 in hepatic impaired and age-matched normals.

MS-325 PK Parameter	Hepatic-Impaired (HI) (n=8)	Age-Matched Normal (AMN) (n=10)	p-value <sup>a</sup>
Cmax (mmol/L)	0.606 ± 0.116	0.700 ± 0.076	0.0564
Tmax (hr)	0.0500	0.0500	1.0
AUC(0-last) (mmol·hr/L)	6.77 ± 2.04	6.51 ± 0.88	0.7234
AUC(0-inf) (mmol·hr/L)	7.06 ± 2.09	6.85 ± 0.96	0.7750
t(1/2alpha) (hr)	1.51 ± 1.80 <sup>b</sup>	0.560 ± 0.079	0.1123
t(1/2beta) (hr)	18.6 ± 6.0	16.1 ± 2.7	0.2698
t(1/2term) (hr)	20.5 ± 7.3	18.8 ± 3.2	0.5373
V(ss) (mL/kg)	172 ± 21	162 ± 14	0.2253
MRT (hr)	24.2 ± 8.2	22.2 ± 3.9	0.4934
Cl(t) (mL/hr/kg)	8.00 ± 3.81	7.42 ± 0.93	0.6433
Cl(r) (mL/hr/kg)	7.00 ± 4.10	6.23 ± 1.01	0.5657
X(u) (%) (14 day)	82.5 ± 7.6	83.6 ± 7.3	0.6981
X(f) (%) (7 day)	2.67 ± 1.88	4.81 ± 3.12	0.0793
X(t) <sup>c</sup> (%) (14 day)	85.2 ± 7.9	88.5 ± 6.4	- <sup>d</sup>

<sup>a</sup> The p-value testing of differences of cohort (HI vs. AMN) means from ANOVA; Tmax from Wilcoxon rank-sum test

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Figure X. Mean plasma concentration-time profile for the AMN group versus the NV Group and a concentration-time profile of the AMN normalized to 0.03 mmol/kg dose.



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Overall, average PK parameters between the hepatic impaired and age-matched normal groups were very similar. In general, the ratio of group means fell in the range of 80-120, and the 90% CI of the group mean ratio was often contained within the 80-120% range. While the  $t(1/2\text{-}\alpha)$  ratio did not fall within this range, differences between the 2 groups in the concentration of MS-325 in the plasma at early times were not notable from the concentration-time curves, and thus this difference is likely an artifact of the analysis. A decrease in the amount of MS-325 excreted fecally was noted for the HI group. However, since the fraction excreted fecally in the normal population is small (average < 5%), the decrease in fecal elimination in the hepatic impaired population is not clinically important.

Greater inter-subject variability in both the PK parameters and in protein binding for the HI group relative to the AMN group were noted, and these results support the notion that hepatic impairment is not necessarily a homogenous set of physiologic conditions. Nevertheless, in general, the average PK parameters in the moderate hepatic impaired population appear similar to those of the age-matched group, and no dose adjustment appears required for pharmacokinetic reasons in these subjects.

One subject (Patient 13) in the HI group showed faster elimination of MS-325. PK parameter values, including % plasma protein binding, were noticeably different. For example, Patient 13 had a  $t(1/2\text{-}\text{elim})$  of 8.9 hours compared to the group mean of 20.5 hours, and had plasma binding of 69.4% at 4 hours compared to 87.5% for the AMN group mean. Patient 13 had an abnormally low serum albumin level at baseline (2.8 g/dL compared to >3.9 g/dL for the rest of the subjects in the HI group). The faster elimination was consistent with the lower protein binding values recorded in this patient. Since the pharmacokinetics, especially distribution and elimination half lives, are strongly

influenced by the non-protein bound fraction of MS-325, a higher non-protein-bound fraction of MS-325 should lead to a faster decrease in plasma concentration, which was observed for Patient 13. Nevertheless, the significance of serum albumin levels on the PK of MS-325 cannot be concluded based on one patient's data. It should be recognized that suspected association between low serum albumin and faster clearance of MS-325 can not be considered as a safety issue. Furthermore, while pharmacodynamics were not measured in this study, the decrease in fraction bound at early time points from patients with lower albumin is not likely to be a significant factor in efficacy either, since a large fraction (>50%) of the agent is bound to albumin even at the earliest post-injection time points, and thus should provide the increase in relaxivity that is required for MR imaging.

This study also examined the PK of a 0.03 mmol/kg dose in the NV group, and compared it to the 0.05 mmol/kg dose in the AMN group. Although pronounced non-linearity in AUC was observed in previous studies at doses >0.05 mmol/kg, comparing the dose of 0.05 mmol/kg and proposed clinical dose of 0.03 mmol/kg, protein binding was not remarkably dose-dependent. Small (10%) differences in protein binding (~70% for the 0.05 mmol/kg dose and ~80% for the 0.03 mmol/kg dose) were observed immediately post-dosing, and both groups approached 90% protein binding by 4 hours. Nevertheless, this concentration-dependent protein binding immediately post-injection does not play a major role in the pharmacokinetics, and nearly dose-proportional results were seen in the comparison of the 0.03 and 0.05 mmol/kg PK parameters.

### Pharmacokinetic Conclusions

Hepatic impaired (HI; Child-Pugh class B) and age-matched normal liver function (AMN) groups had similar group mean PK parameters, including no overall difference in urinary or total elimination of MS-325. Fecal elimination, while small in both groups, was reduced in the HI group relative to the AMN group. Average protein binding was not different between HI (69.4%) and AMN (70.2%) groups immediately following injection, and the amount of MS-325 bound to serum proteins as a function of concentration was similar between the 2 groups.

Despite similar results between group means, 1 HI subject (Patient 13), who had much lower plasma albumin than other patients in both the HI and AMN groups (<2.8 g/dL vs. 3.9-4.6 g/dL), exhibited notably faster plasma elimination relative to the other HI and AMN subjects. This behavior is consistent with the mechanism of action of MS-325 binding to albumin, and does not represent a likely efficacy concern for these patients.

• **Pharmacokinetics were approximately dose proportional in the range of 0.03 to 0.05 mmol/kg MS-325:** healthy normal volunteers (NV cohort) dosed at 0.03 mmol/kg had pharmacokinetic parameter estimates that were approximately dose-proportional to the 0.05 mmol/kg AMN group. Therefore, conclusions from pharmacokinetic studies using either 0.03 or 0.05 mmol/kg doses should be considered equally applicable.

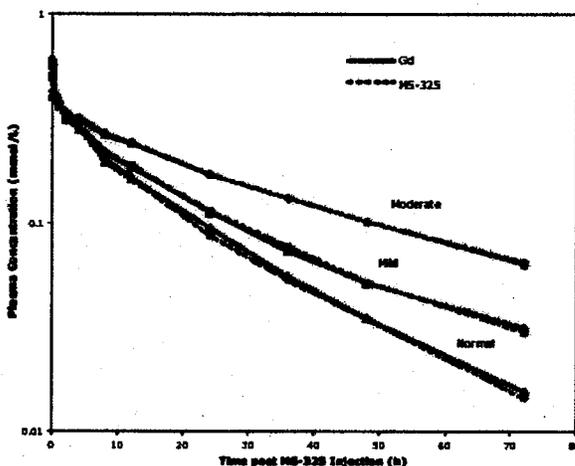
**Study MS-325-07**

A phase II study to evaluate safety and PK of MS-325 in subjects with varying degree of renal insufficiency:

Nineteen serial plasma samples, 10 urine samples, and 10 fecal samples were obtained from subjects in the mild and normal groups from Baseline through 14 days post-MS-325 injection. Additional plasma, urine, and fecal samples were collected from subjects in the moderate group at 21 days post-MS-325 injection. MS-325 was assayed in plasma and urine samples by an HPLC-UV method. Plasma, urine, and feces were also assayed for total Gd using an ICP-AES assay. Plasma concentration of non-protein bound (unbound) MS-325 (i.e., concentration in PUF) was also determined using the ICP-AES assay. The total MS-325 concentration-time data were analyzed for PK parameters using an open two-compartment (bi-exponential) model and a non-compartmental method using WinNonlin. Plasma clearance curves are shown in Figure XI. The following parameters were calculated: AUC, C<sub>max</sub>, t(1/2 $\alpha$ ), t(1/2 $\beta$ ), MRT, V(ss), Cl(t), and Cl(r). Cumulative urinary, X(u), and fecal, X(f), excretion were expressed as percent of administered dose.

The PK samples were assayed for total Gd using a validated ICP-AES method. In brief, a 0.5 mL or 0.5 g sample of plasma, PUF, urine, or fecal homogenate was digested on a hot plate with nitric acid and hydrogen peroxide. The amount of Gd was determined with an ICP spectrometer by comparing the emission of the unknown sample to the emission of the external standard solutions. The results were generated as  $\mu\text{g/mL}$ . Concentration as mmol/L was obtained by dividing by the atomic weight of Gd (157.25 g/mol). The limit of quantitation (LOQ) of Gd was 1  $\mu\text{g/mL}$ . A set of calibration standards (five concentrations each in duplicate) and QC samples (three concentrations each in duplicate) were included in each analysis session to generate a standard curve and to assess assay performance. One blank was run at the beginning and end of each run to check for contamination.

Figure XI. Plasma clearance of MS-325 with time in normal volunteers and patients with mild and moderate renal impairment



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The calculated PK parameters for renal impaired subjects is shown in Table XI  
 The renal clearance decreased substantially in patients with moderate and severe renal impairment. The exposure (AUC) increased almost two fold in patients with moderate and severe renal impairment. The half-life increased from 19 hrs in normal subjects to 49 hours in patients with moderate renal impairment to 70 hours in patients with severe renal impairment.

Table XI. PK profile of vasovist in renal impaired patients

Parameter	Normal	Mild	Moderate	Severe
Cmax, mmol/L	0.71 (26)	0.57 (16)	0.61 (26)	0.67 (21)
AUC(0-inf), mmol*h/L	7.12 (12)	7.52 (28)	12.5 (30)	16.1 (23)
V(ss), L/kg	0.16 (10)	0.17 (12)	0.19 (14)	0.18 (11)
t(1/2term), h	18.9 (14)	22.5 (40)	49.0 (52)	69.5 (43)
Cl(t), mL/h/kg	7.1 (12)	7.0 (29)	4.1 (36)	3.0 (37)
Cl(r), mL/h/kg	5.3 (17)	5.7 (27)	3.0 (34)	2.2 (38)
X(u), % †	74.2 (12)	80.7 (9)	69.1 (11)	65.8 (12)
X(f), % †	6.5 (53)	7.8 (34)	8.5 (58)	13.3 (46)

Note: Renal function was classified using the C-G method: creatinine clearance normal >80 mL/minute; mild impairment >50-80 mL/minute; moderate impairment 30-50 mL/minute; and severe impairment <30 mL/minute.  
 Urine and feces were collected over a 168-hour interval.  
 Source: CSR MS-325-07, Section 11.4.2.2

The determination of PK parameters using two different analytical methods (HPLC-UV assay and ICP-AES) was very comparable (Table XII).

Table XII. Comparison of PK parameters determined by two different analytical methods

Parameter	MS-325 by HPLC-UV Assay			Gd by ICP-AES Assay		
	Normal	Mild	Moderate	Normal	Mild	Moderate
Cmax, mmol/L	0.690, 24	0.577, 20	0.663, 23	0.699, 25	0.556, 19	0.650, 23
Tmax, hr*	0.0170, 88	0.0830, 35	0.0170, 83	0.0170, 74	0.0830, 37	0.0170, 74
V(ss), mL/kg	165.9	177.19	199.18	161.10	173.11	189.13
t(1/2alpha), hr	1.41, 76	4.87, 96	8.42, 97	1.22, 86	3.63, 99	8.21, 103
t(1/2beta), hr	17.2, 17	31.3, 75	64.9, 68	17.0, 14	26.4, 61	54.2, 48
t(1/2term), hr	19.2, 16	34.8, 83	69.3, 36	19.0, 13	26.4, 44	61.4, 47
MRT, hr	22.8, 15	35.0, 60	68.2, 45	23.0, 13	31.8, 45	62.5, 39
AUC(0-inf), mmol*hr/L	6.89, 12	9.49, 45	16.7, 35	7.16, 12	9.08, 41	16.3, 35
Cl(t), mL/h/kg	7.36, 12	6.15, 40	3.44, 42	7.07, 12	6.29, 37	3.51, 40
Cl(r), mL/h/kg	5.59, 13	5.38, 40	3.66, 49	5.39, 17	5.05, 38	2.60, 40
X(u), %	74.2, 12	84.7, 8	64.5, 21	78.6, 11	77.9, 11	67.5, 12
X(f), %	Not Done	Not Done	Not Done	6.38, 53	8.27, 32	11.6, 49

\* The values presented for Tmax are the median and percent coefficient of variation (%CV).  
 Note: Subject group classification based on per-protocol criteria for renal function.  
 Note: One subject in the moderate group was excluded from the mean Gd calculation of X(t) due to no samples recorded from 24 to 120 hours post-dose. One subject in the moderate group was excluded from mean MS-325 calculation of Cl(t), three subjects in the moderate group were excluded from the mean MS-325 calculation of X(u) and one subject in the normal group was excluded from the mean MS-325 calculation of X(u) due to many concentration points not reportable (interference).

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**QTc Evaluation: Study MS-325-09:**

Electrocardiograms were evaluated at a core laboratory by an independent, certified cardiologist and reviewed by the Principal Investigator who determined the clinical significance of any ECG data. Mean Baseline and mean change from Baseline values were calculated for the post-contrast time points. (Study MS-325-09).

Table XIV summarizes the mean Baseline values and mean change from Baseline to One-Hour post MS-325 from two studies, one had no placebo group and ECG time points were defined as Baseline, one-hour post-contrast, and 72-96 hours post-contrast for which 41 patients have data. The protocol was amended on 15 December 1999 and added a placebo group and changed time points to: Baseline, within 45 minutes post-contrast, and 72-96 hours post contrast, for which 197 patients have data.

Table XIV: ECG: Mean Baseline Values and Mean Change from Baseline to One-Hour Post-MS-325

	Placebo mean (sd)	MS-325 (mmol/kg)				
		0.005 mean (sd)	0.01 mean (sd)	0.03 mean (sd)	0.05 mean (sd)	0.07 mean (sd)
Patients	0	9	7	9	9	7
<b>Heart Rate (bpm)</b>						
Baseline	--	71.6 (18.2)	66.3 (10.1)	67.7 (12.1)	68.8 (8.4)	76.3 (15.2)
Change	--	-2.7 (9.3)	-3.7 (6.8)	0.8 (6.1)	1.3 (8.3)	1.9 (10.5)
<b>PR (msec)</b>						
Baseline	--	164.4 (20.4)	160.0 (16.7)	172.4 (21.4)	175.5 (19.8)	153.7 (38.9)
Change	--	-9.8 (13.9)	2.9 (21.3)	1.3 (10.4)	6.0 (10.9)	-5.1 (15.3)
<b>QRS (msec)</b>						
Baseline	--	99.6 (21.3)	99.4 (20.6)	85.8 (7.5)	90.7 (13.1)	92.9 (17.0)
Change	--	-2.7 (3.5)	-0.6 (4.3)	-2.7 (4.5)	-3.1 (6.6)	-2.0 (5.5)
<b>QT (msec)</b>						
Baseline	--	406.7 (57.7)	409.1 (25.9)	405.3 (23.3)	419.3 (27.3)	382.3 (26.4)
Change	--	4.4 (17.9)	10.9 (29.7)	8.4 (12.1)	0.2 (23.2)	-5.7 (21.3)
<b>QTc (msec)</b>						
Baseline	--	435.4 (38.0)	427.3 (16.8)	426.8 (19.5)	446.7 (26.0)	426.7 (19.2)
Change	--	-2.6 (18.0)	-1.0 (23.7)	10.4 (18.9)	3.4 (8.8)	-0.3 (16.1)
Source: Section 16.4.1, Statistical Table S-2.3						

The mean changes from baseline to the 45 minute or 1 hr appear to be small. There is no dose related trend in QT or QTc interval data. The data for the 1 hour post-contrast time point (n=41) comprise QT and QTc intervals for 5 dose groups each. For 4 of the 10 changes in either QT or QTc intervals were a shortening of time interval, and 6 were a prolonging. For the 45 minutes post data (n = 197), the MS-325 0.03 mmol/kg dose group had a mean QTc prolongation of 2.4 msec and the placebo group had a mean QTc prolongation of 3.2 msec. There were 64 patients in this study who had QTc values

greater than 450 msec at any time point (baseline or post-dose). Among all patients who received MS-325 (n = 200), there were 22 patients who had baseline QTc values >450 msec who also had QTc values >450 msec at 45 min or one hour post-dose; 11 patients who shifted from below (at baseline) to above 450 msec at 45 min or 1 hr post-dose; and 15 patients who shifted from above 450 msec at baseline to below 450 msec at 45 minutes to one hour post-dose. For placebo (n=38), there were three patients whose QTc intervals were above 450 msec at baseline and were also above 450 msec at 45 minutes or one hour post-dose; three patients who shifted from below at baseline to above 450 msec at 405 minutes to one-hour; and three patients who shifted from above to below 450 msec. For the high dose groups (MS-325 0.05 mmol/kg and 0.07 mmol/kg combined), there were 10 patients who were above 450 msec at baseline and who were also above 450 msec at 45 min or one hour post-dose; six patients who shifted from below 450 msec at baseline to above 450 msec at 45 minutes or one hour post-dose; and five patients who shifted from above 450 msec at baseline to less than 450 msec at baseline to less than 450 msec at 45 minutes or one hour post-dose. Therefore, at higher doses of MS-325 (0.05 and 0.07 mmol/kg) the frequency of upward shifts to >450 msec was comparable to downward shifts to <450 msec.

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Table XV. Effect of increasing dose of MS-325 on QTc

Parameter	Placebo mean (sd)	MS-325 Dose Groups (mmol/kg)				
		0.005 mean (sd)	0.01 mean (sd)	0.03 mean (sd)	0.05 mean (sd)	0.07 mean (sd)
Patients	38	35	27	30	34	33
<b>Heart Rate (bpm)</b>						
Baseline	66.1(12.3)	70.9(13.3)	70.2 (12.3)	66.2 (12.0)	65.3 (11.1)	68.3 (14.0)
Change	-1.3 (6.6)	-1.3 (10.8)	-2.8 (7.3)	-2.7 (8.2)	-2.6 (7.0)	-0.3 (7.0)
<b>PR (msec)</b>						
Baseline	160.2 (45.6)	165.8 (44.6)	154.2 (52.7)	169.2 (28.3)	158.7 (26.7)	159.5 (39.5)
Change	0.9 (11.5)	-5.2 (28.9)	-6.8 (29.0)	2.5 (17.7)	-1.1 (13.3)	-1.7 (12.3)
<b>QRS (msec)</b>						
Baseline	92.7 (17.5)	92.6 (18.6)	94.8 (16.6)	102.3 (25.3)	93.8 (17.4)	95.1 (15.6)
Change	0.2 (6.0)	-0.8 (5.8)	1.0 (6.2)	0.3 (6.5)	-0.1 (7.3)	1.6 (7.2)
<b>QT (msec)</b>						
Baseline	414.4 (36.1)	403.4 (50.8)	401.0 (34.6)	421.2 (52.6)	416.3 (40.2)	409.2 (43.6)
Change	7.6 (20.5)	7.8 (42.6)	9.6 (15.9)	10.0 (20.0)	11.4 (17.8)	7.0 (20.2)
<b>QTc (msec)</b>						
Baseline	430.0 (18.9)	431.5 (25.4)	428.7 (17.2)	436.5 (33.3)	429.4 (23.2)	429.8 (21.8)
Change	3.2 (14.9)	1.3 (13.9)	0.9 (13.4)	2.4 (16)	2.7 (13.9)	7.8 (10.1)

Source: Section 16.4.1 Statistical Tables S-2.1 and S-2.2

### Drug-Drug Interaction Studies: Study MS-325-06.

A Phase II study was conducted to evaluate the safety of MS-325 in patients with arterial vascular occlusive disease and the safety and pharmacokinetics of MS-325 in patients on warfarin therapy. This study was a single-site, two-arm, single-dose study with open-label pharmacokinetic (PK) arm with two cohorts, PK Reference (REF; receiving study drug alone; 10 patients) and PK Test (TEST; receiving study drug with concomitant warfarin therapy; 10 patients) and a double blind randomized, placebo-controlled Safety Arm with a Safety Treatment cohort (Drug; 10 patients) and a Safety Placebo (Placebo; 10 patients) cohort. The dose of MS-325 injected was 0.05 mmol/kg BW (0.2 mL/kg diluted to 25 mL with normal saline for injection, USP), administered as intravenous bolus at a rate of 1.5 mL/sec, followed by 30 mL normal saline flush. Normal saline for injection was used as a placebo. Variable individual doses of warfarin as prescribed by **patient's physicians were used (recorded dose range 2.5-12 mg 1-4 times a week)**. Effect of warfarin on PK of MS-325 was evaluated by comparing the PK parameters of MS-325 at specific time points post-dose in TEST (MS-325 plus warfarin) and REF (MS-325 alone) groups. The effect of warfarin on PD of MS-325 was evaluated by comparing change in plasma relaxation rate  $D(1/T1)$ , at specific time points post-dose in TEST and

REF cohorts of the PK group. Effect of MS-325 on PK of warfarin was evaluated by comparing the concentrations of R- and S-forms of warfarin in the pre- and post-dose MS-325 plasma samples from the TEST cohort of the PK arm. Effect of MS on PD of warfarin was evaluated by comparing anticoagulant effect (INR values; International Normalized Ratio; prothrombin time normalization) of warfarin in plasma samples obtained pre- and post-MS-325 dose in TEST cohort of PK arm.

RESULTS: PK parameters for MS-325 were derived from plasma concentration-time profiles obtained by the two assay methods, i.e. ICP-AES method for Gd and HPLC/UV method for MS-325. The overall urinary excretion (cumulative 0-168 h) determined using the two assay methods were similar. Gadolinium plasma levels were nearly equivalent to that of MS-325 on a molar basis. Approximately 75 to 80% of administered dose was recovered in urine, indicating that MS-325 is excreted primarily via the kidneys.

Effect of warfarin on PK of MS-325:

The PK variables derived from assay results obtained using ICP-AES method and statistical comparison of the PK variables in the REF and TEST groups are summarized below. The ratio of means along with the mean AUC and C<sub>max</sub> values, expressed on a molar basis, were similar between the TEST and the REF groups. The TEST/REF ratio is expressed as a percent. The TEST/REF mean ratios were contained between 90% confidence intervals. The PK variables in the TEST and REF groups were similar as indicated by the ratio and the confidence intervals.

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Table XVI. Comparison of PK parameters of MS-325 in patients receiving MS-325 alone and MS-325+warfarin

MS-325 (Gd equivalents) Parameter Estimate	REF (MS-325 Alone) (%CV) (n=10)	TEST (MS-325 + Warfarin) (%CV) (n=10)	Ratio (%) (90% CI)
AUC <sub>(0-120)</sub> , µg*hr/mL	1120 (17)	1068 (23)	95.4 (79.9, 111)
AUC <sub>(0-120)</sub> , mmol*hr/L	7.12 (17)	6.79 (23)	95.4 (79.9, 111)
AUC <sub>(0-240)</sub> , µg*hr/mL	1166 (23)	1123 (17)	96.3 (80.9, 112)
AUC <sub>(0-240)</sub> , mmol*hr/L	7.42 (23)	7.14 (17)	96.3 (80.9, 112)
C <sub>max</sub> , µg/mL	87.7 (19)	104 (36)	118 (92.5, 144)
C <sub>max</sub> , mmol/L	0.56 (19)	0.66 (36)	118 (92.5, 144)
t <sub>1/2 (obs)</sub> , hrs	1.72 (65)	0.605 (91)	35.2 (0.00, 75.3)
t <sub>1/2 (beta)</sub> , hrs	20.9 (37)	18.9 (18)	90.6 (68.4, 113)
t <sub>1/2 (terminal)</sub> , hrs	22.2 (27)	20.3 (19)	91.3 (73.7, 109)
MRT, hrs	25.6 (27)	25.5 (19.8)	99.6 (81.5, 118)
V <sub>(ss)</sub> , L/kg	0.172 (9)	0.179 (10)	104 (96.8, 112)
Cl <sub>(0)</sub> , mL/hr/kg	7.07 (23)	7.21 (20)	102 (85.2, 119)
Cl <sub>(0)</sub> , mL/hr/kg	5.22 (24)	5.94 (20)	114 (95.5, 132)
X <sub>r</sub> , % dose	73.1 (11)	81.6 (13)	112 (102, 122)
X <sub>f</sub> , % dose	5.49 (28)	4.42 (57)	80.5 (51.3, 110)

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In vitro studies conducted to assess potential interferences with protein binding of MS-325 indicated that MS-325 does not interact with commonly prescribed medications.

Effect of MS-325 on the PK of Warfarin: Unbound R- and S-warfarin concentrations in plasma remained unchanged following MS-325 administration. The unbound fractions of R- and S-isomers ranged from 0.98 to 1.38% and 0.79 to 1.03%, respectively. These results indicate that treatment with MS-325 had no measurable effect on the fraction of unbound warfarin in plasma. Overall, there were no statistically or clinically significant differences in the levels of MS-325, Gd, and the unbound fraction of MS-325 in plasma between TEST and REF groups in the PK arm of the study.

#### Pharmacodynamic Results:

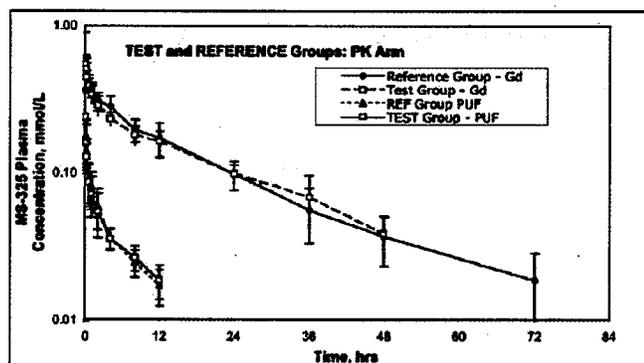
Change in plasma relaxation rate,  $\Delta(1/T1)$ , was not significantly different for the TEST versus REF groups in the PK arm following MS-325 administration.

In conclusion, the pharmacokinetic parameters of MS-325 in patients on concurrent warfarin therapy were not different from those receiving MS-325 alone. Plasma and plasma ultrafiltrate concentrations and derived PK parameters of MS-325 were not affected by the concomitant treatment with warfarin. Absence of clinically relevant influence of warfarin on MS-325 PD was demonstrated by minimal differences in the relaxation rate change,  $\Delta(1/T1)$ , a relevant PD surrogate of MR efficacy, in patients receiving concurrent warfarin versus patients receiving MS-325 alone.

MS-325 demonstrated no clinically significant effect on warfarin kinetics with no differences on the fraction of unbound warfarin or the anticoagulation effect as measured by INR. Adverse events reported by all cohorts were mild and the majority of events

resolved without treatment. There were no statistically significant changes to laboratory parameters, vital signs, physical examinations or electrocardiographic measurements. MS-325 is safe and well tolerated in patients with vascular disease, whether or not the patients are on warfarin therapy.

Figure XII. Plasma clearance of MS-325 in patients with (test) or without (reference) warfarin



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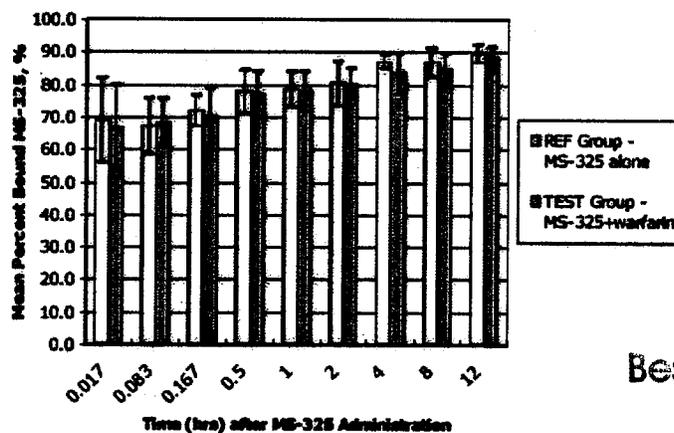
Table XVII. Pharmacokinetic parameters of MS-325 for subjects receiving a single IV dose of MS-325 (0.05 mmol/kg) in the presence or absence of warfarin therapy

Parameter	MS-325 Alone	MS-325 + Warfarin
	Mean (% CV)	Mean (% CV)
C <sub>max</sub> , mmol/L	0.558 (19)	0.661 (36)
AUC(0-inf), mmol <sup>h</sup> /L	7.42 (23)	7.14 (17)
AUC(0-last), mmol <sup>h</sup> /L	7.12 (17)	6.79 (23)
T <sub>max</sub> , h	range: 0.017-0.167	range: 0.017-0.183
t(1/2alpha), h	1.72 (65)	0.605 (91)
t(1/2beta), h	20.9 (37)	18.9 (18)
t(1/2term), h	22.2 (27)	20.3 (19)
MRT, h	25.6 (27)	25.5 (20)
V <sub>(ss)</sub> , L/kg	0.172 (9)	0.179 (10)
Cl(t), mL/h/kg	7.07 (23)	7.21 (20)
Cl(r), mL/h/kg	5.22 (24)	5.94 (20)
X(u), % dose	73.1 (11)	81.6 (13)
X(f), % dose	5.49 (28)	4.42 (57)

In this two-arm study, 22 patients were enrolled in the Pharmacokinetic Arm with 10 patients receiving MS-325 alone and 12 patients receiving MS-325 with concomitant warfarin therapy. The Safety Arm included 20 patients (11 patients receiving placebo and 9 patients receiving MS-325). Of the 42 patients in the study, 33 patients reported a total of 108 adverse events. The majority of AEs 70/108 or 65% of those noted by patients in both study arms (including those in placebo group), were rated as mild in severity, the remaining events (38/108, 35%) were rated as moderate. The most frequently occurring AE that was rated as possibly or probably related to MS-325 was paresthesia (7 events reported by 5 patients).

There were no statistically significant mean changes in laboratory parameters in any group, or at any time point. There were no clinically significant trends in ECG measurements, nor any observed differences in overall ECG results for the MS-325 treated population vs placebo group. There did not appear to be any difference between ECG measurements of TEST (MS-325+warfarin) versus patients receiving MS-325 in the Safety Arm and the REF group (MS-325 alone) in the PK Arm and between Test and Placebo group.

Figure XIII. Mean Percent Binding of MS-325 to Plasma Proteins Following a Single Bolus IV Dose (0.05 mmol/kg) of MS-325 to Subjects in the Presence or Absence of Concurrent Warfarin Therapy



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**Study MS-325-01C:**

Rising single dose (0.01-0.15 mmol/kg), safety, tolerance, and PK study.

In a Phase I (CSR MS-325-01C), double-blind, placebo controlled, rising single-dose, pharmacokinetic study of MS-325 administered as an IV bolus of 5 min IV infusion at 0.05, 0.075, 0.10, 0.075, 0.0.125, 0.15, 0.200 or 0.225 mmol/kg. Groups D, F, and H received a single 30 second IV bolus dose of MS-325 or placebo at 0.050, 0.075 or 0.1 mmol/kg, respectively. Groups E, G, I & J-1 received a single 75 second IV bolus dose of MS-325 or placebo at 0.075, 0.100, 0.15 or 0.125 mmol/kg, respectively. Sixty three healthy male and female volunteers (49 males and 14 females, between the ages of 18 to 43 years) subjects were enrolled in the study (9 subjects per treatment/dose group and 3 placebo). PK blood samples were collected at 0 hour (within 2 hours prior to dose); 2, 5, 10, 20, 30, & 45 minutes; and 1, 2, 3, 4, 6,8, 10, 12, 16, 24, 36, 48, 72, 96, 120, 144 and 168 hours after the end of the 30-second or 75 second IV bolus dose. Albumin binding samples were collected at 0 hr (within 2 hours prior to dose), 20 minutes, and 1, 6, 12 and 24 hours after the end of the 30-second or 75-second IV bolus dose. Plasma relaxation rate ( $\Delta 1/T1$ ) samples were collected at 0 hour (within 2 hours prior to dose), 2, 5, 10, 20, 30 and 45 minutes, and 1, 2 and 4 hours after the end of the 30-second or 75-second IV bolus dose.

The subjects were confined to the clinical site from the time of check-in on day 1 until 168 hours post-dose. Subjects returned to the clinical site for a follow up visit approximately 13 days post-dose. Blood, urine (acid-treated) and stool samples were collected for PK (gadolinium) analysis. Blood samples were also collected for albumin binding analysis and for plasma relaxation time analysis. Urine (non-acid-treated) samples were collected for metabolite (MS-325) analysis. Physical examination, vital signs (including supine blood pressure, pulse, and respiratory rate), 12-lead ECGs, clinical laboratory evaluations and adverse event information were collected throughout the study.

According to the sponsor all 63 subjects completed the study, except for subject 425 and 426 who were discontinued from the study by the principal investigator after collecting their 120 hours post-dose samples (discontinued due to unacceptable behavior). The PK data from all 63 subject obtained are summarized in Table I.

The mean gadolinium C<sub>max</sub> values following the 3-second dose length in Group D, F, and H at the 0.050, 0.075 and 0.100 mmol/kg MS-325 dose levels were 98.3, 129, and 151  $\mu\text{g/mL}$ , respectively. The increase in C<sub>max</sub> was not proportional to the increase in dose level. The doubling of the dose resulted in a 1.5-fold increase in C<sub>max</sub>. The mean gadolinium C<sub>max</sub> values following the 75-second dose length in Group E, G, I and J-1 at the 0.075, 0.100, 0.125, and 0.125 mmol/kg MS-325 dose levels were 134, 162, 186 and 219  $\mu\text{g/mL}$ , respectively. The increase in C<sub>max</sub> was not proportional to the increase in dose level. Doubling of dose resulted in 1.63-fold increase in C<sub>max</sub>.

Similarly, the increase in AUC was not proportional to the increase in dose level. The doubling of dose resulted in about 1.51-fold increase in AUC for the 30 second dose length in Groups D, F, and H at the 0.05, 0.07 and 0.100 mmol/kg and 75-second dose length groups E, G, I and J-1 at the 0.075, 0.100, 0.125 and 0.15 mmol/kg MS-325 dose levels.

The mean gadolinium  $t_{1/2}(\alpha)$  across all dose levels ranged between 0.354 and 0.701. There were no overall statistically significant differences between different doses. Similarly there were no statistically significant differences in mean gadolinium  $t_{1/2}(\lambda_z)$ .

The mean gadolinium CL values for Groups D, F, and H ranged between 0.0083 and 0.0111 L/hr/kg and for the Groups E, G, I and J-1 ranged between 0.0097 and 0.0135 L/hr/kg. There were statistically significant differences in CL across the first dose group ( $p=0.0016$ ) as well as the later dose groups ( $p=0.0046$ ) suggesting that gadolinium clearance was dose dependent in the dose range used in this study.

The mean gadolinium percent of the dose excreted in urine ranged between 74.3% and 84.3% for Groups D, F and H and between 75.5% and 85.5% for groups E, G, I and J-1. These results indicate that gadolinium is mainly excreted in urine after IV dosing. The mean MS-325 percent of dose excreted in urine (as measured by HPLC) ranged between 75.7% and 81.9% for Groups D, F, and H, and ranged between 79.2% and 86.5% for Groups E, G, I and J-1. These values appear comparable, and the percent dose recovered in urine based on the MS-325 data was comparable to those calculated using gadolinium data and indicate that MS-325 is mainly excreted in urine.

The mean gadolinium percent of the dose excreted in feces ranged between 4.67% and 6.86% for Groups D, F, and H, and ranged between 5.86% and 6.63% for Groups E, G, I and J-1. The mean percent free gadolinium 0.333-hour post-dose values ranged between 20.2% and 32.5% for groups D, F, and H, and ranged between 23.4 and 33.8% for Groups E, G, I and J-1. The percent free gadolinium increased as the MS-325 dose level increased across the different dose groups.

**Following the administration of 0.150 mmol/kg as a 75-second IV bolus dose (Dose Cohort Group I) and subsequently 0.125 mmol/kg as a 75-second IV bolus (Dose Cohort Group J-1), changes in the urinary sediment examination (principally appearance of renal tubular cells) were observed in 9 out of 9 subjects and 8 out of 9 subjects, respectively. The sponsor claims, "although a small number of renal cells were observed in the urine sediment of subjects in Group H, I and J-1, the finding did not appear to be due to exposure to study drug but rather to some yet unidentified study procedure."**

**The sponsor further states that, "it is noteworthy that the subjects in which the greatest number of renal cells were observed (i.e., subjects in Groups I and J-1) were infused MS-325 or placebo in a total volume of 90 mL, whereas all other dose groups were infused MS-325 or placebo in a total volume of 45 mL." Due to appearance of renal cells observed in microscopic sediment analysis following MS-**

**325/placebo administration, the sponsor requested that the study be discontinued in order to allow the Sponsor to break the blind and assess the clinical significance of this observation. This study was discontinued following the completion of Dose Cohort Group J-1 (0.125 mmol/kg administered as a 75-second IV dose infusion). As a result, the 5 minutes IV dose infusion escalation sequence was not initiated at all in this study.**

**Analytical Section:**

J. developed and validated the majority of methods used to support the PK studies. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) methods were developed to measure Gd in human plasma, PUF, urine, and feces. Methods using HPLC with UV detection were developed to measure MS-325 in plasma and urine, but due to the inability to reliably recover MS-325 in feces, it was not possible to develop a method for MS-325 in this matrix. All methods at — were performed in accordance with Good Laboratory Practices.

b(4)

For several PK studies, both Gd and MS-325 were measured in plasma and urine samples to assess whether there was any evidence for metabolism. If there were evidence of metabolism of MS-325, less MS-325 than Gd on a molar basis would be found (Report AR009). In addition a detailed analytical study was conducted involving pooled urine samples from the MS325-06 study (Report AR-003). Data from this study show that the HPLC-UV method used for the PK studies compares favorably to potentially more specific methods such as HPLC with MS/MS detection, and HPLC with ICP-MS detection.

ICP with MS detection was used at EPIX Medical, Inc. to measure levels of Gd in urine below the lower limit of quantitation (1 µg Gd/mL) of the — ICP-AES method. This more sensitive method was employed to measure Gd levels in urine in late time points in Study MS-325-16 and in dialysate samples from Study MS-325-18.

b(4)

**Methods Used for Measuring Gadolinium in Human PK Studies and Limits of Quantification**

Matrix	Technique	Laboratory	Reference	Limit of Quantitation
Plasma, PUF, Urine, Feces	ICP-AES	—	6754-104	1 µg Gd/mL (6.4 nmol/L)
Urine, dialysate	ICP-MS/MS	EPIX	EP-ICP-100	0.004 µg Gd/mL (0.025 nmol/L)

b(4)

Inductively Coupled Plasma (ICP) Emission Spectroscopic Method for the Determination of Gd ion from MS-325 in Human Plasma, Plasma Ultrafiltrate, Urine and Feces: A 0.5 mL or 0.5 g sample of plasma, PUF, urine or feces was digested with nitric acid and hydrogen peroxide on a hot plate. The sample was transferred to an appropriate volume

and the amount of Gd was determined with an ICP spectrometer by comparing the emission of the unknown sample to the emission of the external standard solutions.

ICP-MS Procedure for the Determination of Gadolinium and Yttrium (Y) in Liquid Samples (EP-ICP-100): The sample was diluted into calibration range (5 to 300 ng/mL) using nitric acid diluent containing internal standards. Responses were measured for the samples and standards, and the ratio of Gd/Tb is calculated. The sample concentration was determined against a standard curve accounting for dilution of the standard and internal standard.

b(4)

MS-325 (gadofosveset)

Determination of MS-325 in Human Urine using HPLC-MS/MS ( Study No.6754-123):

b(4)

b(4)

Analysis of MS-325 and zinc fosveset in human urine by HPLC with ICP-MS detection (EPIX method HPLC-091):

Determine total gadolinium concentration using ICP-MS (EPIX Method EPICP-100) and dilute sample with water to between 1 and 50 µM. Analysis is performed using HPLC

with ICP-MS detection chromatogram with the ratio of Gd/Tb response is created and the concentration of the sample determined against a calibration curve.

b(4)

Determination of MS-325 isomer in human plasma urine using high-performed liquid chromatography with ultraviolet detection ( Study No 6754-118):

b(4)

b(4)

**Total Zinc:**

ICP-MS procedure for the determination of Zinc in liquid samples (EPIX Method EPICP-101):

The sample is diluted in to the calibration range (5 to 300 ng/mL) using nitric acid diluent containing  $\text{Zn}^{2+}$  as internal standard. The final solution must be at least 2 mL. Zinc responses are measured for the samples and standards, and the ratio of  $\frac{\text{Zn}_{\text{sample}}}{\text{Zn}_{\text{standard}}}$  is calculated. The sample concentration is determined against a standard curve accounting for dilution of the standard and the internal standard.

**b(4)****Zinc Fosvest:**

Analysis of MS-325 and zinc fosveset in human urine by HPLC with ICP-MS detection (EPIX method HPLC-091):

Analysis is performed using high-performance liquid chromatography (HPLC)

with ICP-MS detection. A chromatogram with the ratio of  $\frac{\text{Zn}_{\text{sample}}}{\text{Zn}_{\text{standard}}}$  response is created and the concentration of the sample determined against a calibration curve.

**b(4)****Comparison of Total Gadolinium and MS-325 Concentrations:**

Gadolinium was determined at  $\text{ng/mL}$  using ICP with AES detection and at EPIX using ICP with MS detection. MS-325 was determined at  $\text{ng/mL}$  using HPLC with UV and MS/MS detection and at EPIX using HPLC with ICP-MS detection. Each sample pool was run fifteen times by both methods to generate data sets that could potentially identify a 5% difference between the results with 90% confidence. The QC data generated for the LC/MS/MS run showed a negative deviation of 14.4% for the lowest concentration (15.0  $\mu\text{g/mL}$ ) QC samples and a negative deviation of 12.4% at the mid concentration QC (75  $\mu\text{g/mL}$ ). This deviation is just within the acceptability guidelines outlined for bioanalytical assays. The impact of this bias was to render the statistical comparison meaningless for the lower concentration samples. Table XVIII shows the concentration data for gadolinium measured using ICP-AES and MS-325 measured using LC-MS/MS and LC-UV. A quality examination shows excellent agreement between gadolinium and MS-325 (LC-UV) data and as expected a negative bias at lower concentrations for the MS-325 data generated using the LC/MS/MS technique. Adjustment of the LC-MS/MS data by using the % deviation observed in the QC samples in the same concentration range improves the agreement with the gadolinium values.

**b(4)**

Nonetheless an examination of the data reveals no obvious trend with concentration and the agreement is excellent for the urine pools over the first 48 hours, during which time

majority of the drug (>60%) is excreted in the urine. The ICP-MS and HPLC-ICP-MS data generated at EPIX show good agreement with ~~\_\_\_\_\_~~ results.

b(4)

Table XVIII: Gadolinium and MS-325 Concentration Data generated at ~~\_\_\_\_\_~~ (n=15)

b(4)

Technique	Total [Gd] $\mu$ M		[MS-325] nM (diff. ICP-AES)	
	ICP-AES	HPLC-MS/MS	HPLC-MS/MS (bias corrected)	HPLC-UV
Pool #1 (-12~0h)	0	0	0	0
Control (Pool #1 spiked)	217	198 (-8.5%)	198 (-8.5%)	207 (-4.7%)
Pool #2 (0~3h)	1469	1477 (+0.5%)	1477 (+0.5%)	1475 (+0.4%)
Pool #3 (3~24h)	725	697 (-3.8%)	697 (-3.8%)	705 (-2.8%)
Pool #4 (24~48h)	196.50	190 (-3.3%)	190 (-3.3%)	197 (+0.4%)
Pool #5 (48~72) <sup>1</sup>	93.5	75.9 (-18.8%)	86.6 (-7.3%)	93.6 (+0.2%)
Pool #6 (72~96) <sup>2</sup>	33.5	30.4 (-9.2%)	35.1 (+4.9%)	33.7 (+0.8%)
Pool #7 (96~120) <sup>3</sup>	19.6	15.2 (-22.2%)	17.8 (-9.1%)	18.0 (-8.4%)

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	ICP-AES (Total Gd, $\mu\text{M}$ )	HPLC-MS/MS (MS-325, $\mu\text{M}$ )	HPLC-UV (MS-325, $\mu\text{M}$ )	ICP-MS (Total Gd, $\mu\text{M}$ )	HPLC-ICP-MS (MS-325, $\mu\text{M}$ )
Pool #1 (-12~0)	0	0	0	4.39	0.54
Control (Pool 1 spiked)	217	198	207	202	198
Pool #2 (0~3)	1469	1477	1472	1502	1473
Pool #3 (3~24)	725	697	705	722	695
Pool #4 (24~48)	197	190	197	203	184
Pool #5 (48~72)	93.5	75.9	93.6	95.6	85.6
Pool #6 (72~96)	33.5	30.4	33.7	35.7	30.6
Pool 7 (96~120)	19.6	15.2	18.0	19.8	16.2

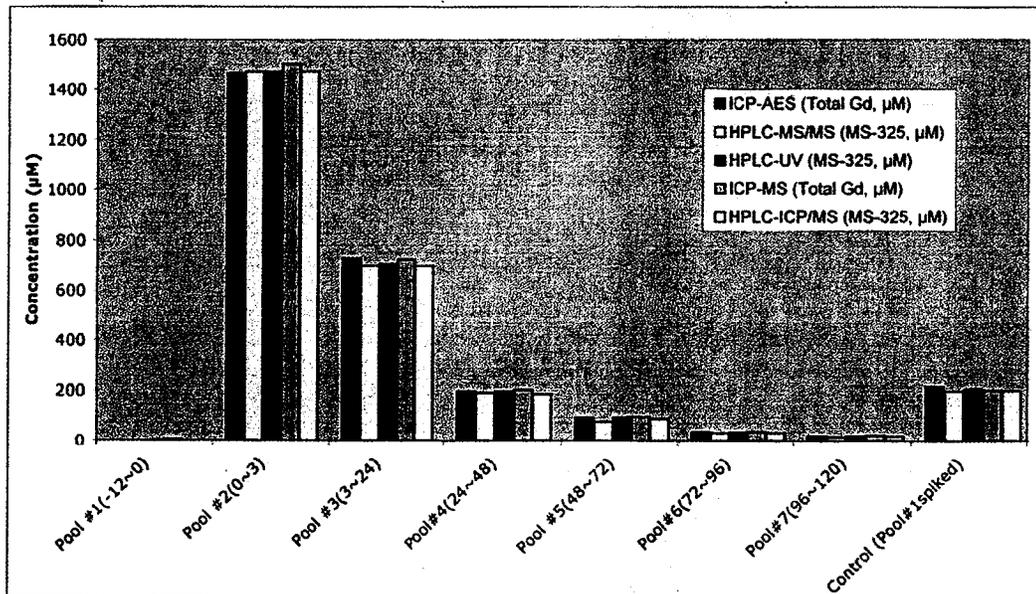


Figure XIV. Comparison of total Gadolinium and MS-325 Concentrations

**Total Zinc and Zinc fosveset Concentrations:**

Total Zinc was determined by ICP-MS and zinc fosveset, the zinc analog of MS-325 (gadofosveset), was measured by HPLC with ICP-MS or MS/MS detection. The results, presented in Table XIX are calculated by dividing the analytical concentration (in  $\mu\text{g}/\text{mL}$ ) by the atomic weight of zinc or the molecular weight of zinc fosveset, as appropriate. The resulting concentration in mM is converted to  $\mu\text{M}$  by multiplying by 1000. — data are provided in the appended analytical report. Appendix 3, — Study No. 6754-130.

b(4)

Table XIX. Total zinc and zinc-fosveset concentration

	ICP-AES (Total Zn, $\mu\text{M}$ )	HPLC-ICP-MS (Zn-fosveset, $\mu\text{M}$ )	HPLC-MS/MS (Zn-fosveset, $\mu\text{M}$ )
Pool #1 (-12-0)	3.28	0.08	0
Control (Pool #1 spiked)	3.53	4.09	1.71
Pool #2 (0-3)	7.73	7.98	18.3
Pool #3 (3-24)	5.48	3.69	3.72
Pool #4 (24-48)	4.50	4.85	5.59
Pool #5 (48-72)	4.66	4.80	4.89
Pool #6 (72-96)	3.90	2.70	3.13
Pool #7 (96-120)	3.62	3.63	3.59

The HPLC-MS/MS method was run concurrent with the measurement of MS-325 at \_\_\_\_\_ and was designed to provide a only a qualitative estimate of the zinc fosveset concentration. These initial results warranted further investigation, particularly the high value obtained from Pool #2 (0-3 h). Total zinc by direct ICP-AES yielded a concentration (7.73  $\mu\text{M}$ ) clearly inconsistent with the amount of zinc fosveset measured at \_\_\_\_\_ (18.3  $\mu\text{M}$ ). The use of HPLC-ICP-MS at EPIX to specifically measure zinc fosveset gave a value (7.98  $\mu\text{M}$ ) comparable to the concentration of total zinc. For other urine pools, the concentration of zinc fosveset is similar to, or lower than, the concentration of total zinc. The levels of zinc fosveset in the various urine pools are similar to those found in urine spiked directly with MS-325 suggesting that a combination of zinc uptake by the free ligand in the formulation, as well as some trans-chelation, occurs in the urine.

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Zinc Ion Concentration from MS-325-16

The sponsor conducted urine zinc excretion in 24-hour pooled urine samples (microgram/24 hr) in normal volunteers (NV) and age matched normal liver function. Zinc excretion in both groups rose in first 24 hours, and returned to baseline by 72 hours post-dose. For 0.03 mmol/kg NV Cohort, there is an increase in urine zinc excretion during 24 hours after dosing; the mean increase was 281  $\mu\text{g}/24$  hours (Table XX). The data for AMN Cohort indicated an increase in urine zinc excretion at 0.05 mmol/kg dose. However, the sponsor claims that one patient had abnormally high zinc excretion (11,011 at  $\mu\text{g}/24$  hours). Thus, it is clear from this limited data that there is a likelihood of in-vivo dissociation.

Table XX. Urine zinc excretion in 24-hour pooled urine samples ( $\mu\text{g}/24$  hours)

Time Point	NV Group (0.03 mmol/kg)			AMN Group (0.05 mmol/kg)		
	N	Mean (SD)	Mean Change (SD)	N	Mean (SD)	Mean Change (SD)
Baseline	10	556 (295)	-	10	534 (276)	-
24 hours post-dose	10	837(416)	281 (170)	10	1856 <sup>a</sup> (3226)	1302 (3032)
72 hours post-dose	10	622 (393)	66 (161)	10	622 (207)	68 (119)

The sponsor claims that there were no remarkable changes in serum zinc levels by 48-72 hours and the short term increase in zinc concentration was not clinically relevant.

#### CLINICAL STUDY I

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The primary efficacy analysis was based on the sensitivity, specificity, and accuracy for the detection of clinically significant stenosis ( $\geq 50\%$ ) in all target vessels from patients in the 0.03 mmol/kg dose group in the Phase III studies. Results for sensitivity and specificity are summarized in Table XXI. In Study MS-325-12, all three readers demonstrated improvements from pre-contrast to post-contrast in sensitivity (range: 6-19%) and specificity (range: 8-20%). The improvements in sensitivity were statistically significant for two readers ( $p < 0.001$ ), and the improvements in specificity were statistically significant for all three readers ( $p < 0.001$ ). Additionally, all three readers showed statistically significant improvements in accuracy (range: 8-20%;  $p < 0.001$ ). In Study MS-325-13, all three readers demonstrated improvements from pre-contrast to post-contrast in sensitivity (range: 22-31%) and specificity (range: 9-12%). The improvements in both parameters were statistically significant ( $p < 0.001$ ) for all three readers, and all three readers also had statistically significant improvements in accuracy (range: 11-13%;  $p < 0.001$ ). I

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Overall, for nine of the 12 readers in the Phase III studies, there was a statistically significant improvement in sensitivity, for 12 of 12 readers, there was a statistically significant improvement in specificity, and for 11 of 12 readers, there was a statistically significant improvement in accuracy.

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Table XXI. Comparison of sensitivity and specificity of pre and post-MS-325 contrast images.

Study (Disease Type) MRA Reader Variable	Number of Patients [2]	Number of Vessels [2]	Post- contrast (%)	Pre- contrast (%)	Difference (%) [3]	p-value [4]
<b>MS-325-12 (AIOD)</b>						
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
<b>MS-325-13 (AIOD)</b>						
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
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

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Primary Efficacy Analysis from Phase III Study MS-325-12:

The primary efficacy analysis was based on the blinded reader assessments for sensitivity, specificity, and accuracy for the post-contrast MRA procedure compared with pre-contrast MRA using the XRA as the SOR. The primary analysis was based on data from the ITT patient population, which was defined as those patients who received study drug, underwent post-MS-325 administration MRA and had XRA.

Of the 268 patients in the ITT population, 251 had XRA data with at least one interpretable vessel after the adjudication process in the blinded read. Patients with at least one significantly diseased vessel (>50% stenosis) form the sensitivity evaluable population; patients with at least one non-diseased vessel (<50% stenosis) form the specificity evaluable population. Patients with at least one interpretable vessel in the SOR form the accuracy evaluable population. Therefore, patients could contribute vessels for

analysis in both the sensitivity and specificity evaluable populations. The results are shown in Table XXII.

Sensitivity was calculated as the number of correctly identified abnormal vessels (i.e., stenosis >50%) from the MRA scans (TP) divided by the total number of abnormal vessels as determined by XRA (TP+NAP+FN). Specificity was calculated as the total number of correctly identified normal vessels (i.e., stenosis <50%) from the MRA scans (TN) divided by the total number of normal vessels as determined by XRA (TN+NAN+FP). Accuracy was calculated as the number of correctly diagnosed vessels from the MRA scans (TP+TN) divided by the total number of vessels with interpretable XRA images (TP+NAP+FN+TN+NAN+FP), and it was used as the overall measure of sensitivity and specificity in an aggregate sense. Sensitivity, specificity, and accuracy were evaluated for each blinded reader as well as the institutional readers. The analysis for the blinded read data was considered the primary analysis.

Table XXII. Primary efficacy analysis of pre and post-MS-325 IV administration in phase III study

Parameter	Number evaluated by XRA		Post-MS-325 (%)	Pre-MS-325 (%)	Difference (%)	p-value
	Patients	Vessels				
Accuracy	251	1646				
Reader A			83.8	73.2	10.6	<0.001
Reader B			90.3	82.2	8.1	<0.001
Reader C			90.3	70.6	19.7	<0.001
Sensitivity	140	237				
Reader A			80.2	62.0	18.1	<0.001
Reader B			73.0	66.7	6.3	0.060
Reader C			60.8	41.8	19.0	<0.001
Specificity	250	1409				
Reader A			84.5	75.1	9.4	<0.001
Reader B			93.2	84.8	8.4	<0.001
Reader C			95.3	75.4	19.9	<0.001

All three readers showed clinically and statistically significant improvements in diagnostic efficacy for MS-325-enhanced MRA compared to non-contrast MRA. In addition, all three readers (individually) showed an improvement in accuracy, sensitivity and specificity. This difference was statistically significant for all readers for accuracy and specificity, and significant for two of the three readers for sensitivity.

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**Uninterpretable Images:**

Uninterpretable images are defined as those images having at least one uninterpretable side. Interpretable sides (right and left) of the MRA image are those that had three or more interpretable vessels for a given reader. All other image sides ( $\leq 2$  vessels interpretable per side) were classified as uninterpretable.

	Post-MS-325 (%) n=256	Pre-MS-325 (%) n=256
Reader A	1.2	17.6
Reader B	0.4	4.7
Reader C	0.4	21.9

The results for the average absolute difference between pre- and post-contrast MRA and XRA are summarized below. All readers showed better absolute agreement (i.e., smaller differences) versus XRA for the MS-325-enhanced MRA compared to non-contrast MRA.

	MS-325-Enhanced (%) n=1646	Non-contrast (%) n=1646
Reader A	16.2	25.3
Reader B	11.4	18.2
Reader C	11.7	28.0

**4.3 Consult Review (including Pharmacometric Reviews)**

N/A

**4.4 Cover Sheet and OCPB Filing/Review Form**

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Office of Clinical Pharmacology and Biopharmaceutics New Drug Application Filing and Review Form				
General Information About the Submission				
Information		Information		
NDA Number	21-711	Brand Name	Vasovist (Gadofosveset)	
OCPB Division (I, II, III)	II	Generic Name		
Medical Division	HFD-160	Drug Class		
OCPB Reviewer	Christy S. John, Ph.D.	Indication(s)	Contrast agent for magnetic resonance angiography	
OCPB Team Leader	Young Moon Choi, Ph.D.	Dosage Form	Pale yellow solution	
		Dosing Regimen	0.12 ml/kg (0.03 mmol/kg)	
Date of Submission	December 22, 2003	Route of Administration	Intravenous injection	
Estimated Due Date of OCPB Review		Sponsor	Epic Medical	
FDDEA Due Date		Priority Classification		
Division Due Date				
Clin. Pharm. and Biopharm. Information				
	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments if any
<b>STUDY TYPE</b>				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X			
<b>I. Clinical Pharmacology</b>				
Mass balance:				
isozyme characterization:	X			
Blood/plasma ratio:	X			
Plasma protein binding:	X			
Pharmacokinetics (e.g., Phase I) -				
<b>Healthy Volunteers-</b>				
single dose:	X	2		
multiple dose:	X			
<b>Patients-</b>				
single dose:	X	1		
multiple dose:	X			
Dose proportionality -				
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:	X	1		
In-vivo effects of primary drug:	X	1		
In-vitro:				
Subpopulation studies -				
ethnicity:				
gender:	X			
pediatrics:				
geriatrics:	X			
renal impairment:	X	1		
hepatic impairment:	X	1		
PD:				
Phase 2:				
Phase 3:				
PK/PD:				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:				
Population Analysis -				
Data rich:				
Data sparse:				
<b>II. Biopharmaceutics</b>				
Absolute bioavailability:	N/A			
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				

<b>Bioequivalence studies -</b>				
traditional design: single / multi dose:	N/A			
replicate design: single / multi dose:				
<b>Food-drug interaction studies:</b>				
Dissolution:	N/A			
(IV)VC:				
<b>Bio-waiver request based on PCS</b>				
<b>PCS class</b>				
<b>iii. Other QBR Studies</b>				
<b>Genotype/phenotype studies:</b>				
<b>Chronopharmacokinetics</b>				
<b>Pediatric development plan</b>				
<b>Literature References</b>				
<b>Total Number of Studies</b>				
<b>Fiability and QBR comments</b>				
	<b>"X" if yes</b>	<b>Comments</b>		
<b>Application fiabile ?</b>	X			
<b>Comments sent to firm ?</b>				
<b>QBR questions (key issues to be considered)</b>	<p>The key issues to be considered for this application would be dose linearity, dose justification and PK/PD relationship.                  Another key aspect of this study is protein binding as the mechanism of action of MS-325 is through extensive protein binding. The sponsor claims that the high protein binding characteristic of MS-325 increases the relaxivity four-fold as compared to non-conventional non-protein binding gadolinium contrast agents.</p>			
<b>Other comments or information not included above</b>				
<b>Primary reviewer Signature and Date</b>	Christy S. John, Ph.D. 2/9/04			
<b>Secondary reviewer Signature and Date</b>	Young Moon Choi, Ph.D. 2/9/04			

CC: NDA 21-711, HFD-850 (P. Lee), HFD-860 (M. Mehta)

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this page is the manifestation of the electronic signature.**

/s/  
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Christy John  
12/3/04 03:40:17 PM  
BIOPHARMACEUTICS

Young-Moon Choi  
12/3/04 03:46:07 PM  
BIOPHARMACEUTICS