Pre-clinical and Clinical Safety Studies of CMX-2043: A Cytoprotective Lipoic Acid Analogue for Ischaemia–Reperfusion Injury

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Abstract: CMX-2043 is an α-lipoic acid analogue targeted to reduction of cellular injury and organ damage due to ischaemia–reperfusion injury (IRI). It has been shown to be effective in a rat model of cardiac IRI. The studies here reported evaluate its safety and pharmacokinetic profile in preparation for human clinical studies in procedures associated with IRI. Safety and tolerability were tested in standard pre-clinical *in vitro* and animal models and in a Phase 1 human clinical trial. CMX-2043 did not bind to a wide range of receptors and specific targets at approximately 4 μg/mL (10 μM). It was not mutagenic by Ames assay, did not produce chromosome aberrations in Chinese hamster ovary (CHO) cells, and was negative for clastogenic potential. Toxicological studies in rats including both single and 14-day repeat intravenous doses and in dogs (single intravenous dose) with a 2-week recovery period were conducted. The NOAEL in rats and dogs was 30 and >10 mg/kg, respectively. No serious adverse events were reported in a placebo-controlled, sequential dose escalation Phase 1 clinical trial. The low toxicity in the pre-clinical studies and the absence of adverse events in the Phase 1 trial have supported investigation of CMX-2043 in a human efficacy trial.

Prevention and treatment of ischaemia–reperfusion injury (IRI) is a major unmet medical need. In such injury, local inflammation, production of free oxygen radicals, alterations in calcium homeostasis and initiation of apoptotic cell death are all believed to contribute to potentially serious pathological and functional consequences [1,2]. IRI occurs in many organ systems, including heart, kidney, liver and brain. Although numerous treatment options have been evaluated, none has attained clinical acceptance [3]. Thus, there remains a need for an easy to use, safe and efficacious therapeutic agent for prevention or reduction of IRI. CMX-2043 (α-*N*-[(R)-1,2-dithiolane-3-pentanoyl]-L-glutamyl-L-alanine) is an α-lipoic acid (LA) analogue targeted to IRI. The LA moiety was used as the backbone structure to confer lipophilicity and antioxidant properties, and for its known ability to activate cellular protective mechanisms [4,5]. CMX-2043 reduces cardiac IRI in a rat model and is more effective in this model than LA itself. Its mechanism of action is consistent with established cell survival pathways (Lader AS, BaguisiA, Casale R, Kates SA, Beeuwkes III R, manuscripts in preparation) .

The safety of the CMX-2043 parent molecule LA has been extensively validated. It is well tolerated in human beings and has been shown to be extremely safe in multiple *in vitro* and animal studies [6]. Consistent with its attractive safety profile, LA in oral (600 mg tablet [7]) and parenteral (Thioctacid® 600 T [8]) formulations has been found to be effective for complications associated with diabetes [9,10]. This back-ground supported the development and testing of a family of LA analogues as potential therapeutic agents. From these, based on biochemical and animal efficacy studies [11], CMX-2043 was selected as the lead candidate for further investigation. Results of pre-clinical safety studies and a Phase 1 human trial are reported here.

Methods and Materials

The chemical structure and properties of CMX-2043 and lipoic acid are listed in table 1.
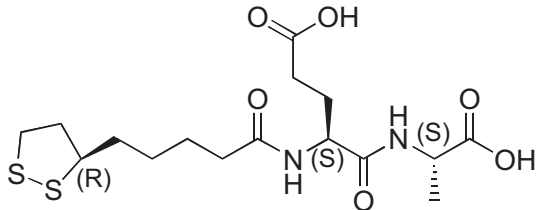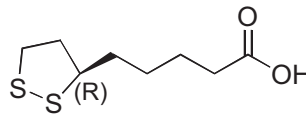
Dose solution preparation and analysis. For non-clinical studies, the free acid of CMX-2043 was added to a solution that included sufficient 0.5 N NaOH for its neutralization, together with water and NaCl in quantities calculated to produce isotonicity and desired concentration. Analysis of dosing solution samples was conducted in compliance with GLP regulations by BASi (Evansville, IN, USA) using a high-performance reversed-phase liquid chromatography with UV detection (HPLC-UV). The concentration of CMX-2043 in the dosing solution was determined using a CMX-2043 external working standard. The cycle/run time for the HPLC analysis was an 8-min. isocratic gradient. CMX-2043 eluted in this HPLC method at ~3.5 min. Prior to testing for rat and dog toxicology studies, analysis of the dosing solution showed that measured doses ranged from 91% to 94% and from 97% to 100%, respectively, of nominal range.

For clinical studies, CMX-2043 drug product was prepared under cGMP conditions at Bio-Concept Laboratories, Inc. (Salem, NH, USA) at a concentration of 10 mg/mL in a buffered sodium phosphate saline solution in a pH range of 6.8–7.6. CMX-2043 was added to a solution containing sterile water for injection, sodium chloride, sodium phosphate dibasic and sodium hydroxide, and the pH of the solution was adjusted using 1 N sodium hydroxide or 1 N hydrochloric acid to 7.1 ± 0.1. The concentration of CMX-2043 in the drug product was

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Table 1.

Chemical properties of CMX-2043 and lipoic acid.

	CMX-2043	Lipoic acid
Chemical structure		
Molecular formula	$C_{16}H_{26}N_2O_6S_2$	$C_8H_{14}O_2S_2$
Molecular weight	406.52	206.33
CAS number	910627-26-8	1077-28-7
pKa[1]	3.50	4.52
Optical rotation[2]	+36.1°	+115 ± 5°

[1]Theoretical calculation using MarvinSketch version 5.5.0.1 (Budapest, Hungary) with pKa plugin.
[2]EtOH, 25°C, 10 mg/mL.

determined using a CMX-2043 external working standard. The cycle/run time for the HPLC analysis was a 40-min. linear gradient. CMX-2043 eluted in this HPLC method at ~14.0 min.

In vitro adsorption, distribution, metabolism and excretion. In vitro adsorption, distribution, metabolism and excretion (ADME) studies were conducted at Cerep (Redmond, WA, USA) according to their institutional standard operating procedures (SOP) (http://www.cerep.fr/cerep/users/pages/catalog/profiles/catalog.asp). Solution property assays, human plasma protein binding solubility in PBS at pH 7.4, solubility and CLogP, were determined by HPLC-UV/VIS detection.

Metabolism and plasma stability were assessed using human liver microsomes and human plasma, respectively, at 1 μM (4.065 μg/mL) and 5 μM (2.0325 μg/mL) concentrations, respectively, of CMX-2043. CYP inhibition was assessed using various substrates at 10 μM (4.065 μg/mL) of CMX-2043 (a complete list of the conditions is provided in the Supporting Information).

Receptor screen. Commercially available *in vitro* receptor screens were performed by Cerep to determine the possible interaction of CMX-2043 with a wide range of receptors and enzymes. For these studies, CMX-2043 was dissolved in DMSO at a concentration of 4.065 μg/mL (10 μM) and diluted with the specific assay buffer. Specific ligand binding to the molecular target was defined as the difference between the total binding and the non-specific binding determined in the presence of an excess of unlabelled ligand. The results are expressed as percentage of control specific binding and as percentage inhibition of control specific binding. In each experiment, the respective reference compound was tested concurrently with CMX-2043 in order to monitor assay validity. Molecular targets that were identified as hits were followed up to determine IC_{50} using concentrations of 1, 3, 10, 30 and 100 μM.

Genotoxicity. Mutagenicity (*in vitro* assay) and clastogenicity (*in vivo* and *in vitro*) assays were conducted at MicaGenix (Greenfield, IN, USA) according to the company's SOPs [12–18].

Ames assay. Suspension of Salmonella Typhimurium tester strains TA98, TA100, TA1535 and TA1537 and the Escherichia coli tester strain WP2uvrA plus S9 mix or plain buffer without S9 were incubated for 20 min. at 37°C with CMX-2043 at concentrations of 0 (vehicle control), 313, 625, 1250, 2500 and 5000 μg/plate. After 20 min., agar was added to the cultures and the contents of the tubes were thoroughly mixed and poured onto the surface of Petri dishes containing standard bacterial culture medium. The plates were inverted and incubated at approximately 37°C for 48–72 hr prior to counting.

Chromosome aberration assay in vitro. Chinese hamster ovary (CHO) cells were cultured *in vitro*, exposed to CMX-2043 at progressive 1:1 dilutions from 2030 μg/mL (5000 μM) in the 4-hr assay with S9 and progressive 1:1 dilutions from 4060 μg/mL (10,000 μM) in 4-hr and 19-hr assays without S9. Exposure levels were selected based on concurrent cytotoxicity data. After exposure, the cells were arrested in metaphase. Slides were prepared, stained with Giemsa and microscopically examined for chromosomal aberrations.

Micronucleus assay (chromosome aberration assay in vivo). Groups of 10 mice (5/sex) were given intravenous doses of CMX-2043 at 0 (vehicle only), 70, 140 and 280 mg/kg/day for 2 days or a single oral dose of cyclophosphamide monohydrate (CP) at 50 mg/kg. Bone marrow was harvested 24 hr after the last dose to evaluate potential bone marrow toxicity and measure the frequency of micronucleated polychromatic erythrocytes (MPCEs).

CV and respiratory safety pharmacology studies.

hERG assay. Human ether-a-go-go-related gene (hERG)-related potassium current studies were conducted at MPI Research (Kalamazoo, MI, USA) using a standardized whole-cell patch-clamp method [19–21]. HEK293 cells were co-transfected with hERG cDNA and G418-resistant gene incorporated into a modified pCDNA3 plasmid. Transfectants were maintained under constant selection pressure incorporated into the culture media. Cells were harvested and plated 1 day prior to electrophysiological evaluation. After whole-cell patch-clamp and demonstration of stable hERG current for at least 2 min., CMX-2043 at concentrations of 40.65, 121.96, 406.5, 1219.62 μg/mL* (100, 300, 1000, 3000 μM*) (*upper limit of solubility) was applied in physiological salt solution for 2.3–5.7 min. Currents were recorded with a Multiclamp 700A amplifier and pClamp software (Molecular Devices, Sunnyvale, CA, USA).

Cardiovascular and respiratory safety pharmacology study of CMX-2043 administered by intravenous injection to telemetered beagle dogs. Studies were conducted at Charles River Laboratories, Worcester, MA, USA. Six dogs (3/sex) instrumented with arterial and venous vascular access ports and a telemetry device were given single intravenous infusions (6 mL/kg at a rate of 6 mL/min) of 0.9% sodium chloride (placebo) for injection on day 1 and repeat doses of CMX-2043 (8.5, 20.5 or 60 mg/kg) on days 4, 11 and 18, respectively. Clinical observations were recorded at least once daily starting on day 1, and body-weights were measured before each dose and on day 25. Blood pressure (systolic, diastolic and mean arterial), heart rate, respiratory rate, core body temperature and electrocardiogram were monitored via telemetry from 24 hr before to 24 hr after each dose. Shortly before and at 5, 15 and 30 min. and 1, 4 and 24 hr after dosing, arterial blood samples were collected to measure pH, pCO_2, pO_2, oxygen saturation (sO_2) and bicarbonate (HCO_3^-). Venous blood samples were collected to measure CMX-2043 concentration and evaluate systemic exposure at pre-dose and at 5, 15 and 30 min. and 1, 4 and 24 hr after dosing on days 1, 8, 15 and 22. Toxicokinetic analysis was performed using WinNonlin version 5.1 (Pharsight, Cary, NC, USA) by the calculation of standard parameters. The animals were returned to the testing facility's telemetry colony at the conclusion of the study.

Non-clinical toxicology.

Single-dose toxicology in rats and dogs. Studies were conducted at BASi. Groups of animals (Sprague–Dawley rats, 10/sex; beagle dogs, 6/sex) were given single intravenous infusions of CMX-2043 (rats: 0, 30, 100 or 200 mg/kg; dogs: 0 or 10 mg/kg) on day 1. Rats were dosed at a volume of 10 mL/kg. Solutions were administered at a rate no faster than 0.2 mL/min into a surgically placed catheter in the femoral vein. Dogs were dosed at a volume of 6.0 mL/kg at a rate which did not exceed 6.0 mL/min through an indwelling intravenous catheter. The catheter was flushed with up to 1.0 mL of normal saline. One half of the animals (rats 5/sex/group; dogs 3/sex/group) were euthanized and necropsied on day 2 to evaluate toxicity. The remaining animals were observed for two weeks and then euthanized and necropsied on day 16 to evaluate recovery from any CMX-2043-related toxic effects that might have occurred. Parameters evaluated were clinical signs, changes in body-weight and food consumption, ophthalmic effects, haematological parameters, coagulation, serum chemistry, gross pathology, organ weights and histopathology (adrenals, brain, epididymides, heart, kidney, liver, lung, ovaries, pituitary, prostate, spleen, testes, thymus (region), thyroids/parathyroids, uterus). Test article systemic distribution (toxicokinetics) was also evaluated using WinNonlin version 5.1 by the calculation of standard parameters from blood samples collected at 10 min., 30 min., 1, 2, 4, 8 hr and 24 (±2 min.) after dosing.

In an additional study, groups of six dogs (3/sex) were given single intravenous infusions of CMX-2043 at dose levels of 25 or 75 mg/kg. Two dogs/sex served as a control group and were given vehicle only. Parameters evaluated were clinical signs, changes in body-weight and food consumption, ophthalmic examinations, haematology, coagulation and serum chemistry, and systemic exposure to CMX-2043 (blood samples collected at pre-dose, 10 and 30 min. and 1, 2, 4, 6, 8 and 10 hr (±2 min.) after dosing).

14-day repeat dose toxicology in rats. This study was conducted at Reliance Life Sciences (Mumbai, India). Groups of 12 Wistar rats (6/sex) were given intravenous infusions via the tail vein of CMX-2043 (5, 15, 30 mg/kg) each day for 14 consecutive days. The dose volume was 5.0 mL/kg body-weight throughout the study.

On day 15, animals were euthanized and necropsied to evaluate toxicity. Additionally, recovery groups of 12 rats (6/sex) were given repeat intravenous infusions of CMX-2043 for 14 consecutive days at dose levels of 0 (vehicle only) or 30 mg/kg. On day 29, animals were euthanized and necropsied to evaluate recovery from any CMX-2043-related effects. Parameters evaluated were clinical signs and mortality, changes in body-weight and food consumption, ophthalmic effects, haematology, clinical chemistry, urinalysis, gross pathology, organ weights and histopathology (as described above).

Randomized, placebo-controlled, sequential dose escalation study in healthy adult male and female human volunteers. The study was conducted by NorthWest Kinetics (Tacoma, WA, USA). The Aspire Institutional Review Board (IRB) reviewed and approved the study protocol, informed consent form (ICF), and Investigator Brochure before volunteers were screened for entry to the study. Each volunteer gave informed consent and was evaluated by the principal investigator to assess study eligibility before undergoing any study procedures or receiving the study drug. Enrolled volunteers met all inclusion criteria and no exclusion criteria (see Supporting Information). Study volunteers were randomized to sequential cohorts receiving 20 mg (Cohort 1), 60 mg (Cohort 2), 150 mg (Cohort 3) and 300 mg (Cohort 4) of CMX-2043 by a 6 ± 1 min. intravenous infusion. In each cohort, six volunteers were randomized to receive CMX-2043, and two volunteers were randomized to receive placebo. A second 60-mg cohort was added to address anomalies (see Results). The study included five scheduled visits to the study centre. The screening visit took place between day -28 and -1. Admission for inpatient confinement, qualification, enrolment, dosing and observation was from day -1 to the evening of day 2. A day 4 visit, safety review visit (day 8 ± 1 day) and final visit (day 30 ± 2 days) were also performed. Additional visits for screening procedures and/or follow-up were scheduled as necessary. No individual volunteer received drug more than once in this study.

Two volunteers (1 for CMX-2043 and 1 for placebo) were dosed at 20 mg 1 hr apart on the first day of the initial cohort (Cohort 1). Remaining volunteers in Cohort 1 were dosed on the following day in intervals of at least 30 min. For all subsequent cohorts, after the first volunteer in the cohort was dosed, subsequent volunteers were dosed of at least 20-min. intervals. Safety assessment included physical examination, vital signs, electrocardiogram (ECG) and clinical laboratory evaluation. Blood samples were collected prior to dosing and at 1, 5, 10, 20, 30 and 60 min. and 1.5, 2, 4, 6, 8, 16 and 24 hr after each dose for the measurement of plasma CMX-2043 concentration. Urine specimens were pooled into collections for time intervals of 0–2 hr, 2–4 hr and 4–16 hr after dosing.

Results

In vitro ADME.

The CMX-2043 *in vitro* ADME profile is shown in table 2. CMX-2043 was approximately 59.8% protein bound at a concentration of 4.065 μg/mL (10 μM). Other solution properties included solubility of >100 mg/mL (PBS, pH 7.4) and ClogP of 0.27. CMX-2043 at a concentration of 0.4065 μg/mL (1 μM) was stable in human liver microsomes for 60 min. at 37°C. CMX-2043 was stable in human plasma for at least 60 min. at 37°C at a concentration of 2.032 μg/mL (5 μM). At a concentration of 4.065 μg/mL (10 μM), CMX-2043 had an insignificant or no inhibitory effect on the human cytochrome P450 enzyme subtypes CYP1A2, CYP2C9, CYP2E1, CYP2B6, CYP2C19, CYP3A4, CYP2C8, CYP2D6, CYP3A5.

Receptor and kinase screen.

The receptors and specific targets assayed with CMX-2043 at a concentration of 4.065 μg/mL (10 μM) are listed in table 3. Only N-type voltage-gated Ca^{2+} channel showed a significant

Table 2.

In vitro adsorption, distribution, metabolism and excretion (ADME) profile of CMX-2043.

Properties	Test	Result
Solution properties	Human plasma protein binding	59.80%
	Solubility (PBS, pH 7.4)	>100 mg/mL
	ClogP	0.27
Metabolism		Parent remaining (%)
	Plasma stability (5 μM, 2.032 μg/mL), 60 min at 37°C	100
	Human metabolic stability (liver microsomes) (1 μM, 0.40 μg/mL), 60 min at 37°C	95
CYP inhibition profile (10 μM)	CYP1A2, CYP3A4, CYP3A5	No inhibition detected
	CYP2B6	
	CYP2C8, CYP2C9, CYP2C19	
	CYP2D6	
	CYP2E1	

Table 3.

Classes of receptors and specific receptors assayed against CMX-2043 at 4.065 μg/mL (10 μM).

Receptor class	Specific targets
Adenosine	A1, A2A, A2B, A3
Adrenergic	α1, α2, β1, β2, β3
Angiotensin	AT1, AT2
Benzodiazepine	BZD
Bombesin	BB (non-selective)
Dopaminergic	D1, D2S, D3, D4.4, D5
N-Formyl peptide	fMLP
Vasoactive intestinal peptide	PACAP
Platelet activating factor	PAF
Prostanoid	TXA2
Purinergic	P2X, P2Y
Vasopressin	V1a, V1b, V2
Ion channels	
Calcium	L-type (DHP site), L-type (diltiazem site), L-type (verapamil site), N-type (EF hand)
Potassium	K_{ATP}, K_V, SK
Sodium	Na (site 2)

inhibition. A follow-up study indicated that CMX-2043 had an IC_{50} of 2.032 μg/mL (5 μM) when competing against ω-conotoxin binding to EF hand (structure responsible for Ca^{2+} binding) on the alpha1B subunit of the N-type voltage-gated Ca^{2+} channel.

The kinases, receptors and the corresponding specific biological targets assayed with CMX-2043 at a concentration of 4.065 μg/mL (10 μM) are listed in table 4. CMX-2043 did not compete for binding to any of the kinases or receptors listed in tables 3 and 4 at 4.065 μg/mL (10 μM).

Table 4.

Classes of kinases and receptors and specific biological targets assayed against CMX-2043 at 4.065 μg/mL (10 μM).

Molecule class	Specific targets
Ca/calmodulin-dependent kinases	AMPKα, CaMK2α, CaMK4, CHK1, CHK2, MAPKAPK2
CDK/MAPK/GSK	CDC2/CDK1, CDK2, ERK2, IKKβ, JNK1, JNK2, p38α, p38γ
Cyclic nucleotide or phospholipid regulated kinases	Akt1, p70S6K, PDK1, PKA, PKCα, PKCγ, ROCK2, RSK2
Cytosolic tyrosine kinases	Abl, BMX, Brk, CSK, FAK, Fes, JAK3, Lck, Lyn, Src, ZAP70
Receptor tyrosine kinases	c-Met, EGFR, EphB4, FGFR1, VEGFR1, FLT-3, IGFR1, IRK, VEGFR2, PDGFRβ, TRKA
Homolog of yeast sterile kinase	MAP2K1
Signal transduction proteins	Adenylyl cyclase, guanylyl cyclase, PLC
ER calcium channels	IP3, RyR
Glutamate receptors	AMPA, glycine, kainate, NMDA, PCP
Muscarinic receptor	M (non-specific)
Phosphodiesterases	PDE1, PDE2, PDE3, PDE4, PDE5, PDE6

Genotoxicity.

CMX-2043 was negative for mutagenicity in the Salmonella Typhimurium–Escherichia coli reverse mutation (Ames) assay when tested at up to 5050 μg/plate. CMX-2043 was negative for clastogenicity in CHO cells in the presence and absence of the mammalian metabolic activation system (S9) at 2030 μg/mL (5000 μM). Intravenous administration of CMX-2043 at up to the maximum tolerated dose level (140 mg/kg/day) for two consecutive days did not induce the formation of micronuclei in bone marrow polychromatic erythrocytes (PCEs) in CD-1 mice. CMX-2043 was judged to be negative for clastogenic potential.

Cardiovascular and respiratory safety pharmacology.

CMX-2043 produced no inhibition of hERG-mediated potassium current at its limit of solubility (3000 μM; 1220 μg/mL) when assessed using whole-cell patch-clamp electrophysiological methods.

Administration of CMX-2043 by intravenous injection to telemetered Beagle dogs at 8.5, 20.5 or 60 mg/kg did not adversely alter respiratory rate, core body temperature, blood gases (pCO_2, pO_2, O_2 saturation or HCO_3^-) or electrocardiographic parameters (PR, QRS, QT and QTc). Overall, electrocardiogram (ECG) waveforms appeared qualitatively normal after administration at all dose levels (see Supporting Information for a list of minor effects of administering CMX-2043 at 60 mg/kg dose level).

The no-observable adverse effect level (NOAEL) for cardiovascular effects of intravenously administered CMX-2043 in dogs was thus considered to be 60 mg/kg. This dose in dogs produced an average C_{max} of approximately 329 μg/

mL (809 µM) and an average AUC$_\infty$ of approximately 218 h·µg/mL.

Single and 14-day repeat dose toxicology in rats.
There were no unscheduled deaths and no test article-related clinical observations from a single intravenous administration of CMX-2043 at 30, 100 and 200 mg/kg. Rats tolerated single intravenous doses of CMX-2043 at up to 100 mg/kg. The only effects at 100 mg/kg were slightly reduced platelet count and slightly increased (approximately 2×) aspartate aminotransferase and alanine aminotransferase activities in females. At 200 mg/kg, toxicity in both sexes was characterized by weight loss, impaired coagulation, increased numbers of circulating white blood cells of all types and liver-related clinical chemistry and histopathological changes. All effects were noted within 24 hr after dosing and were reversible within 14 days after dosing (see Supporting Information for detailed description).

The NOAEL was considered to be 30 mg/kg in females and 100 mg/kg in males, which produced an average C_{max} of 18 µg/mL (44 µM) and 193 µg/mL (475 µM) in females and males, respectively, and an average AUC$_\infty$ of 6.2 and 70 h·µg/mL in females and males, respectively. As a follow-up to the single intravenous dose study, a 14-day repeat dose study was conducted. Based upon the results of the single dose, 30 mg/kg was selected as the highest dose with lower doses of 1/2 (15 mg/kg) and 1/6 (5 mg/kg) of the highest dose.

In the expanded 14-day repeat 5, 15 or 30 mg/kg intravenous dose study in Wistar rats with a 2-week recovery period, there were no unscheduled deaths, no drug-related clinical observations (behaviour and growth), no drug-related effects on any of the parameters measured and no adverse effects on haematology and clinical chemistry parameters. At the cellular level, spontaneous/incidental lesions observed in animals treated at 30 mg/kg were comparable to control animals.

Single-dose toxicology in dogs.
In the expanded single 10 mg/kg intravenous dose of CMX-2043 in dogs with a 2-week recovery period, there were no drug-related effects on any of the parameters measured. In the additional study, there were no CMX-2043-related effects on mortality, ophthalmic status, clinical observations, haematology, body-weight or food consumption. The only effects of a single intravenous administration of CMX-2043 at 75 mg/kg in the dogs were slight, short-lived increases in activated partial thromboplastin time (APTT) and possibly prothrombin time (PT). Based on these results, the intravenous NOAEL was considered to be 10 mg/kg, which produced an average C_{max} of 19 µg/mL (44 µM) and an average AUC$_\infty$ of 12.2 h·µg/mL.

Toxicokinetics.
The toxicokinetic profile of intravenous CMX-2043 over a wide range of dose levels was evaluated in rats and dogs. Plasma drug concentration was measured at intervals after administration, and non-compartmental pharmacokinetic analysis was performed to determine standard parameters. These parameters are summarized in the Supporting Information.

In rats, CMX-2043 plasma concentrations decayed in a bi-exponential fashion at the 100 and 200 mg/kg dose levels and were measurable for 1 hr after the end of dosing for the 30 mg/kg dose, 3 hr after the 100 mg/kg dose and for 4 hr after the 200 mg/kg dose. Concentrations increased with an increase in dose level, with the highest CMX-2043 concentrations achieved after the 200 mg/kg dose where the mean concentration at approximately 10 min. after the end of dosing was 485 µg/mL in males and 400 µg/mL in females.

Both C_{max} and AUC$_\infty$ values increased more than proportionally to the dose level. The $t_{1/2}$ for the 30 mg/kg dose was 0.16 hr in males and 0.14 hr for females. Half-lives were longer for the 100 and 200 mg/kg doses, at 0.55 and 0.57 hr and 0.97 and 0.81 hr in males and females, respectively. Consistent with the disproportionate increase in the AUC$_\infty$ values, the clearance values for CMX-2043 were similar for the 100 and 200 mg/kg dose levels, ranging from means of 1.4 and 1.9 L/hr/kg in males and females, respectively. The V_{ss} values at the 100 and 200 mg/kg dose levels were similar in magnitude and relatively small, ranging from 0.41 to 0.61 L/kg.

In dogs, CMX-2043 plasma concentrations were measurable for up to 2 hr after the 10 mg/kg dose, for 4–8 hr after the 25 mg/kg dose and for up to 10 hr after the 75 mg/kg dose. Concentrations increased with dose, with the highest CMX-2043 concentrations achieved after the 75 mg/kg dose where the mean (±S.D.) concentration at 30 min. after the end of dosing was 156 ± 8 µg/mL and 164 ± 3 µg/mL for male and female dogs, respectively. Plasma CMX-2043 concentrations decayed in a monoexponential fashion in most dogs given 10 mg/kg, but some dogs given 25 mg/kg and all dogs receiving 75 mg/kg displayed a biexponential profile, with the break point occurring between 4 and 10 hr after dosing. Systemic exposure (C_{max} and AUC$_\infty$) to CMX-2043 increased with dose level in a linear fashion and, in general, in proportion to the increase in dose. $t_{1/2init}$ was short at all dose levels, averaging 0.28–0.45 hr, and tended to increase with an increase in dose. $t_{1/2\lambda z}$ averaged 0.28–2.87 hr with the longest half-lives observed at 75 mg/kg, likely due to the ability to measure CMX-2043 for a longer period of time in plasma at this highest dose level.

The volumes of distribution (V_{ss}) at steady-state were consistent in both species, ranging from 0.41 to 0.61 L/kg in rats and 0.32 to 0.38 L/kg in dogs.

Randomized, Placebo-Controlled, Sequential Dose Escalation Study in Healthy Adult Male and Female Human Volunteers

A total of 40 volunteers were enrolled in the study. All volunteers who were randomized completed the study and no volunteer was discontinued early from the study. The second cohort (60 mg) was repeated to assess whether anomalies seen in the coagulation tests in two of the volunteers were due to technique or drug, thereby doubling the planned enrolment into that cohort. The Safety Review Committee concluded that the anomalies in coagulation tests were due to technique,

specifically the placement of venous blood sampling cannulae in peripheral veins leading to slow blood withdrawal and haemolysis. The cannulae were placed more proximally in later cohorts.

The mean age of the 40 volunteers enrolled in the study was 28 years (range 18–44); the mean BMI was 25 (range 20–31); the mean height was 70 inches (range 64–75); and the mean weight was 175 pounds (range 128–224). Cohorts were balanced with regard to these four demographic parameters. Most (67%) of the volunteers were Caucasian, with 22% black. Both races were represented similarly in all five cohorts. The remaining 10% of volunteers included two Asians, one American Indian and one 'Other'. There was only one female in the study. Demographic and baseline characteristics are summarized in the Supporting Information.

After intravenous bolus administration of single, escalating doses of 20, 60, 150 and 300 mg, the mean terminal-phase half-life ($t_{1/2}$) of CMX-2043 was short, ranging from an average of 0.86 hr at the 20-mg dose to 1.45 hr at the 300-mg dose. The AUC_∞ ranged from 583 to 16483 h·ng/mL and increased with increasing dose in a slightly greater than dose proportional manner resulting in a slight decrease in clearance with increasing dose. The clearance (CL) of CMX-2043 was high and ranged from 19.3 L/hr for the 300-mg dose to 35.3 L/hr for the 20-mg dose. The volume of distribution of the central compartment (V_1) was relatively small, approximately 6 L, and the apparent volume of distribution at steady-state (V_{ss}) was 2 to 3 times greater and decreased slightly with increasing dose.

Most of the urinary excretion occurred in the first 4 hr and approximately 30–45% of the administered dose was excreted unchanged in the urine over a 16-hr collection interval.

Human safety.

Six of 10 volunteers (60%) on placebo reported a total of 10 treatment-emergent adverse events (TEAEs) and 17 of 30 volunteers (57%) on study drug reported a total of 32 TEAEs. For the volunteers that received 20 mg, 60 mg and 150 mg of CMX-2043 study drug, four (66.7%), 10 (83.3%) and 3 (50%), respectively, reported TEAEs. There were no TEAEs reported in the 300-mg dose group and no serious TEAEs reported for any of the volunteers. All TEAEs were considered mild in intensity except for four events categorized as moderate. These were considered by the investigator 'definitely not related' to study medication. No volunteer discontinued the study for any reason. All TEAEs by organ class and preferred term are summarized in the Supporting Information.

Seven TEAEs, each occurring in one volunteer who received study medication, were considered at least possibly related to study medication: dry mouth, polyuria, fatigue, orthostatic lightheadedness, chest discomfort, pain in injection site, decreased neutrophil count. All AEs were mild and resolved without treatment. The TEAE of chest discomfort was considered 'closed' at 58 hr after dosing.

Discussion

The clear result of the studies here reported is evidence of remarkably low toxicity of a compound that is effective against IRI in an animal model (Baguisi A, Stewart K, Casale R, Lader AS, Kates SA, Beeuwkes III R, manuscript in preparation). In the expected clinical setting, intravenous administration is the route of choice. This low toxicity is consistent with the pharmacology of α-lipoic acid (LA), the parent molecule of CMX-2043 [22].

α-Lipoic acid has shown no evidence of mutagenicity in the Ames test and no genotoxicity in a mouse micronucleus assay after a single oral administration of 825 mg/kg [22]. The LD_{50} value for LA administered orally was greater than 2000 mg/kg [22]. In a 4-week subchronic rat toxicity study, no signs of toxicity or differences in clinical symptoms or pathology occurred with daily doses of 31.6 or 61.9 mg/kg administered by oral gavage. The non-observable adverse effect level (NOAEL) of LA has been reported to be 61.9 mg/kg/day [22]. In a chronic toxicity/carcinogenicity study with LA in the diet of Sprague–Dawley rats at doses up to 180 mg/kg/day, no carcinogenicity or target organ toxicity was observed; the NOAEL was 60 mg/kg/day [23]. In dogs, the MTD is 126 mg/kg and an oral LD50 has been reported as 400–500 mg/kg [24]. The safety of LA has also been demonstrated in multiple human clinical studies, including SYDNEY, SYDNEY 2, ALADIN I, II and III and NATHAN I and II [25–29].

α-Lipoic acid has been reported to be 10 times more toxic in cats than in human beings, dogs or rats [30]. Cats are not typically used in GLP FDA-regulated toxicity studies, and CMX-2043 has not been administered to this species.

In the receptor screen, CMX-2043 did not compete for binding to any of the kinases or receptors listed in tables 3 and 4 at approximately 4 µg/mL (10 µM) except competing against ω-conotoxin binding to alpha1B subunit of the N-type voltage-gated Ca^{2+} channel. The significance of this inhibition is unclear because the ratio of CMX-2043 concentration to that of the specific ligand (conotoxin) was 10^6 to 1. However, this interaction is presently in further study and evaluation.

Negativity for Ames and clastogenicity in CHO cells was at the limit of solubility and for the formation of micronuclei in bone marrow at the maximum tolerated dose of CMX-2043. As an acute clinical therapy, these results suggest that the risk of carcinogenicity and reproductive toxicity is extremely low.

A comparison of LA and CMX-2043 toxicity in animals and human beings is presented in table 5. The expanded single-dose toxicity studies in rats identified NOAELs of 30 and 100 mg/kg in females and males, respectively. In dogs, the NOAEL was >10 mg/kg.

Effects of CMX-2043 on coagulation were noted at the higher dose levels in rats and dogs and were characterized by prolonged APTT in both species, with slightly prolonged PT in dogs and low platelet count in rats. Effects on coagulation were short-lived and fully reversible and returned to normal within 24 hr in dogs and within 14 days in rats. Effects potentially related to liver toxicity were also noted at highest dose levels in rats and dogs and were fully reversible within

Table 5.
Comparison of lipoic acid and CMX-2043 toxicity in animals and human beings.

	Lipoic acid	CMX-2043
Non-clinical toxicity		
Ames test	None	None
Micronucleus	None, mouse 825 mg/kg	
Toxicity		
Mouse	LD50 > 2000 mg/kg oral	Not performed
Rat	28 day none, 61.9 mg/kg oral	NOAEL 30 mg/kg in females, IV NOAEL 100 mg/kg in males, IV
Dog	MTD = 126 mg/kg LD50 (oral) = 400–500 mg/kg	NOAEL not identified at 10 mg/kg, IV
Cat	MTD = 13 mg/kg	Not performed
Human beings		
Toxicity	>300 mg oral	>300 mg IV
Treatment	300 mg oral	2.4 mg/kg

14 days. This observation is consistent with oral administration to rats of high doses of LA (121 mg/kg bw) causing slight alterations in liver enzymes as well as histopathological effects on the liver and mammary gland [22].

Studies with CMX-2043 administered to telemetered dogs monitored for cardiovascular and respiratory changes suggest little potential risk of adverse effects on cardiovascular or respiratory function. The minor decrease in arterial blood pressure and heart rate observed at the high dose of 60 mg was transient and not considered clinically significant. Negativity for hERG inhibition at the limit of solubility of CMX-2043 and the cardiovascular and respiratory NOAEL of 60 mg/kg in dogs represented exposure at levels which far exceeded the doses and maximum concentrations planned and found in the Phase 1 clinical study.

The toxicokinetic studies showed conventional exponential profiles in both rats and dogs. The volumes of distribution were slightly greater than the extracellular fluid (ECF) volume, but less than total body water (TBW) volume, in both species. These V_{ss} values likely reflect the polar nature of CMX-2043. The small volumes of distribution and high clearance values likely account for the plasma CMX-2043 concentrations being in the μg/mL range and the half-lives being relatively short.

Allometric scaling was performed using the clearance (CL) estimates (in mL/min) and body-weights (in kg) (Supporting Information). These data, although limited to two species, predicted human clearance of 440 mL/min or 0.352 L/hr/kg. Use of this CL value and an average body-weight of 60 kg yielded a predicted area under the curve (AUC) in human beings of 0.76 hr·μg/mL at the 20 mg starting dose in the Phase 1 trial (the actual AUC_{0-t} in healthy human beings was found to be 0.58 hr·μg/mL at the 20 mg dose).

These pre-clinical safety pharmacology and toxicity studies in rats and dogs indicated that CMX-2043 would be safe when administered to human beings at low dose levels. The expanded single-dose toxicity studies in rats and dogs identified NOAELs of 30 and 10 mg/kg, respectively. Using the FDA methodology for calculating the human equivalent dose (HED) based on body surface area for a 60-kg person (FDA Draft Guidance Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers, 2005), these NOAELs are equivalent to human doses of 292 and 324 mg, respectively. Based on this standard, the non-clinical safety studies conducted with CMX-2043 supported the safety of a starting dose level of 20 mg, which is less than 1/10th of the lower NOAEL-based HED.

Potential adverse effects in human beings that might occur in especially sensitive individuals or at high doses could include blood coagulation or liver function effects, both of which were observed in the animal studies, albeit at dose levels much higher than those anticipated to be used in human trials. Furthermore, both types of toxicity could be readily detected in human beings, should they occur, allowing appropriate intervention if required. LA itself has been found to be very safe at the doses recommended for clinical use in human beings. Rare adverse effects in human beings include allergic skin conditions [24,31], nausea and vomiting [27], headaches [31,32] and hypoglycaemia in patients with diabetes [33,34]. LA is well absorbed by the oral route, although in human beings LA has been shown to have limited bioavailability of about 30% due to hepatic first-pass metabolism [32].

The CMX-2043 NOAEL levels in rats and dogs subsequently proved to be approximately 14 and 16 times higher, respectively, than the Phase 1 trial starting (20 mg) dose for the lightest volunteer (58 kg; 0.34 mg/kg) and 24 and 27 times higher, respectively, than the low mg/kg dose for the heaviest volunteer (102 kg; 0.2 mg/kg), within an acceptable safety margin. The CMX-2043 NOAEL in rats and dogs was approximately 10% higher and lower, respectively, than the Phase 1 trial 300-mg dose for the lightest volunteer (60 kg; 5.0 mg/kg) and about half of the exposure in mg/kg to the heaviest volunteer (97 kg; 3.1 mg/kg), within an acceptable safety margin.

The Phase 1 human study described here was designed to assess the safety and tolerability of single IV doses of 20, 60, 150 or 300 mg of CMX-2043 compared with placebo. All doses studied were particularly well tolerated. There were no serious adverse events (SAEs), severe TEAEs or TEAEs that led to discontinuation of any volunteer. In fact, there were no premature discontinuations in the study for any reason. There was no relationship of dose with TEAE frequency or intensity. This is underscored by the absence of AEs at the highest dose, 300 mg, 15 times the lowest dose of 20 mg. There were insufficient data to draw meaningful conclusions regarding the effect of age, gender or race on the frequency or severity of TEAEs. The pre-defined dose-limiting toxicity (DLT) criteria for CMX-2043 were not met. A maximum tolerated dose (MTD) was not established in this study because all doses were well tolerated. In the absence of a dose response for total TEAEs or any single TEAE, it could not be concluded that any TEAE observed in this study was definitely treatment

related. There were no clinically significant effects on individual findings or mean changes to suggest an effect of study drug on vital signs, ECG, physical examination findings or clinical laboratory tests.

Urinary excretion data indicated that renal elimination of unchanged CMX-2043 was an important clearance component, because approximately 30–45% of the administered dose was excreted unchanged in the urine over a 16-hr collection interval. Most of the urinary excretion occurred in the first 4 hr. The volume of distribution of the central compartment (V_1) was relatively small, approximately 6 L, indicating that CMX-2043 initial distribution approximates plasma volume. The V_{ss} was 2 to 3 times greater, approaching ECF volume, and decreased slightly with increasing dose.

The short plasma half-life and relatively rapid clearance suggests that if a drug effect were to depend on its presence in the circulation, frequent administration or prolonged infusion may be required. On the other hand, if CMX-2043 were to act by the activation of cell regulatory systems having long-term effects, a single or perhaps only a short duration administration may suffice to produce a therapeutic effect. Indeed, in rats, a single intravenous dose of 10 mg/kg provides significant and long duration for protective effects on IRI despite a 10-min. plasma $t_{1/2}$ (manuscript in preparation).

The results reported here from both the pre-clinical and clinical studies provide strong evidence that, like its parent molecule LA, CMX-2043 can be safely administered to patients. This attractive profile supported further evaluation of intravenous administration of CMX-2043 in patients potentially at risk of ischaemia–reperfusion injury.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. HPLC Chromatogram of Clinical Dosing Solution.

Figure S2. Linear and Logarithmic Mean Plasma CMX-2043 Concentration-Time Data for Male and Female Rats.

Figure S3. Linear and Logarithmic Mean Plasma CMX-2043 Concentration-Time Data for Dogs.

Figure S4. Allometric Scaling for CMX-2043.

Figure S5. Mean Plasma CMX-2043 Concentration *vs.* Time Plots.

Table S1. Experimental Conditions for CYP Inhibition, Metabolic Stability and Plasma Stability.

Table S2. Summary of CMX-2043 Inhibition with Human Cytochrome P450 Enzyme Subtypes.

Table S3. Summary of Ames Assay.

Table S4. Percentages of Cells with Chromosome Aberrations and Aberration Frequency in CHO Cells.

Table S5. Micronucleus Assay - Micronucleus Frequency and PCE/NCE Ratios.

Table S6. CMX-2043 hERG Current Inhibition Data.

Table S7. Mean Body Weights and Body Weight Changes in Rats.

Table S8. Selected Mean Haematology Parameters in Rats.

Table S9. Selected Mean Clinical Chemistry Parameters in Rats.

Table S10. Selected Mean Organ Weights in Rats.

Table S11. Mean Body Weights and Body Weight Changes in Dogs.

Table S12. Selected Mean Haematology Parameters in Dog.

Table S13. Selected Mean Clinical Chemistry Parameters in Dog.

Table S14. Selected Mean Organ Weights in Dogs.

Table S15. Selected Coagulation Parameters in Dogs.

Table S16. Summary of Mean CMX-2043 Toxicokinetic Parameters in Rats and Dogs.

Table S17. Subject Demographic and Baseline Characteristics-Safety Population.

Table S18. Summary of Non-compartmental Pharmacokinetic Parameters.

Table S19. All Treatment-Emergent Adverse Events.

Appendix S1. Materials and Methods.