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Absence of developmental or reproductive toxicity in rats for MB-102, a fluorescent tracer agent for point-of-care measurement of glomerular filtration rate

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ABSTRACT

The fluorescent tracer agent MB-102 was designed for the direct, real-time measurement of glomerular filtration rate. Previous studies, both *in vitro* and *in vivo* (rats, rabbits and dogs) have assessed single dose toxicity, phototoxicity, local tolerance, hERG channel changes, mutation, chromosomal aberration, micronuclease assays, and CNS and cardiovascular safety. The resulting safety/toxicology profile allowed FDA clearance to conduct Phase I and II human clinical studies. Herein we report on maternal toxicity and potential effects on embryo-fetal development and toxicokinetics of MB-102 administered daily via intravenous (bolus) injection into pregnant rats during organogenesis gestation day 6–17. Mortality, clinical observations, body weight, food consumption, reproductive performance, necropsy, and cesarean section findings were assessed. Blood samples were evaluated toxicokinetically. No significant findings were noted in any endpoints. The only clinical findings were skin, eye or pelage discoloration in the two higher dose groups, which were considered related to the color and fluorescent properties of MB-102 and deemed non-adverse. Exposure, assessed by C_{max} and $AUC_{(0\text{-}6)}$, increased in a dose-dependent manner from 9 to 225 mg/kg/day. Thus, intravenous administration of MB-102 was not associated with any adverse developmental or reproductive toxicities in pregnant rats.

1. Introduction

Glomerular filtration rate (GFR) is the standard for measurement of renal function (National Kidney Foundation, 2002) by nephrologists and the medical community. However, the current clinical determination is only an estimate of GFR, called eGFR. Its basis is a single-point measurement of serum creatinine (SCr) from a blood draw. One of several empirically derived equations converts SCr to eGFR. It is well-known in the nephrology community that this methodology has many issues (Michael A Ferguson and Waikar, 2012; Inker et al., 2012), including a 24–72 h time delay in response to a kidney insult (Endre et al., 2011), a lack of sensitivity, as 50% of kidney function may be lost before abnormal results are observed, factors not related to kidney function may skew results, and finally the conversion equations are ensemble averages and not necessarily applicable to a particular individual.

The use of exogenous fluorescent tracer agents offers the possibility of overcoming these issues by providing a methodology for a true direct measurement of GFR at the point-of-care. This has been explored by several groups over the past 20 years (Chinen et al., 2008; Rabito et al., 2005; Schock-Kusch et al., 2009; Yu et al., 2007), with none being successfully translated to the clinic as of yet. MB-102 is a novel fluorescent tracer agent that has been rationally designed to enable direct measurement of GFR, thus filling a serious unmet medical need. MB-102 has previously exhibited characteristics essential for accurate real-time measurement of GFR in several animal models (Poreddy et al., 2012; Rajagopalan et al., 2011), and has been clinically demonstrated to be a GFR agent in subjects with normal renal function (Dorshow et al., 2015). In first-in-human clinical studies, this agent has been shown to be well-tolerated in subjects over the range of renal function from normal to Stage 4 chronic kidney disease (Dorshow et al., 2015, 2017).

Previously, MB-102 was evaluated in a first-tier and next-tier battery of *in vitro* and *in vivo* safety/toxicity studies. The sum-total of these studies (Bugaj and Dorshow 2015; Dorshow and Bugaj 2019) resulted in negligible demonstrable pathological test article concerns, leading to a safety/toxicity profile that was deemed sufficient by the FDA to begin a first-in-human clinical study, and subsequently Phase I and Phase II human clinical trials.

The experiments described below assess maternal toxicity and

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potential effects of MB-102 on embryo-fetal development in pregnant rats following intravenous administration.

2. Materials and methods

2.1. Fluorescent tracer agent

MB-102 is a compound in the pyrazine class, with a molecular weight of 372.3 (chemical name 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine, Fig. 1). It has light absorption and emission peaks at 445 nm and 560 nm respectively (Rajagopalan et al., 2011).

MB-102 is formulated with phosphate-buffered saline for intravenous administration. Properties of MB-102 and its clinical formulation have been recently published (Shieh et al., 2020).

2.2. Study design: dosing schedule and toxicokinetic evaluation

The purpose of this study was to evaluate maternal toxicity and potential effects of MB-102 on embryo-fetal development. In addition, toxicokinetics were determined for the test article when administered daily via intravenous (bolus) injection to pregnant rats during the period of organogenesis (Gestation Days [GD] 6 through 17) as shown in Table 1.

Approximately 0.5 mL of blood was collected from the jugular vein of three animals/group/time point into tubes containing sodium heparin anticoagulant, as shown in Table 2.

Toxicokinetic (TK) parameters listed in Table 3 were estimated, as data permitted.

2.3. Animal selection and Dose Administration rationale

Rats were chosen for this study because they are sensitive to a number of agents known to cause reproductive or developmental toxicity. Rats historically have been used in safety evaluation studies of this type and are recommended by appropriate regulatory agencies. The Sprague-Dawley rat was selected based on availability of historical control data and susceptibility to known developmental toxicants.

All procedures in the protocol were in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare (Covance Laboratories, Salt Lake City, Utah) and were approved by the local Institutional Animal Care and Use Committee (IACUC).

The doses were chosen because they are 1, 10, and 25-fold the planned human equivalent dose (HED) of 1.5 mg/kg, in accordance with the FDA's guidance *Developing Medical Imaging Drug and Biological Products Draft Guidance for Industry* (Food and Drug Administration, 2004).

Animals were checked twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress. Abnormal observations were recorded as they were observed. Detailed observations were made on GD 4, 6, 9, 12, 15, 18, and 21 for each toxicity animal. Abnormal findings or an indication of normal was recorded. Cageside observations were made for toxicity animals once daily during the dosing interval (GD 6 through 17), approximately 1 h post-dose, based on the last animal dosed for each group. Any abnormal findings were recorded. Unscheduled observations were recorded as they were observed.

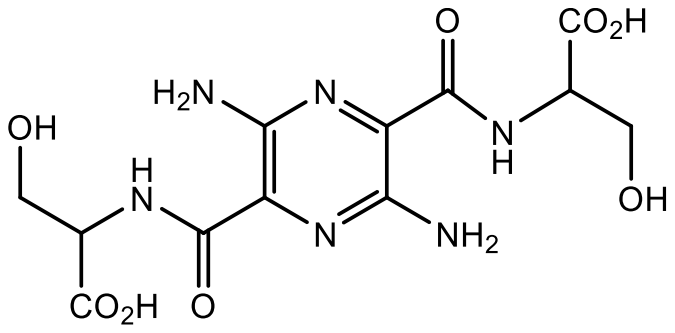

Fig. 1. Structure of 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2 hydroxyethyl] carbamoyl}pyrazine, MB-102.

Toxicokinetic animals were weighed on GD 0, 4, and 6 through 17. Toxicity animals were weighed on GD 0, 4, 6 through 18, and 21. The animal supplier provided GD 0 body weights for entry into the computer data collection system. For toxicity animals, food consumption was measured on GD 4, 6, 9, 12, 15, 18, and 21.

Pregnancy status was determined. If no fetuses were in utero and if implantation sites were not apparent when pressing the uteri between glass plates, then the uterus was placed in ammonium sulfide solution to confirm the absence of pregnancy. Live fetuses were sacrificed with an appropriate barbiturate followed by exsanguination.

2.4. Maternal necropsy

Cesarean sections were performed on GD 21 for toxicity animals. Animals were sacrificed via carbon dioxide inhalation, followed by exsanguination. Pregnancy status was determined, uterine contents were examined, and the number of implantation sites was recorded, when present. When implantation scars were not apparent when pressing the uteri between glass plates, the uterus was placed in ammonium sulfide solution to confirm the absence of pregnancy. The uterus from each pregnant animal was excised, weighed, and examined for the number and placement of live and dead fetuses, the number of early or late resorptions, and any abnormalities. The right and left ovaries from each pregnant female were examined for the number of corpora lutea. Each animal was examined macroscopically for abnormalities of the external features of the carcass; external body orifices; cervical, abdominal, and thoracic viscera; organs; and tissues; macroscopic abnormalities were noted.

2.5. Fetal examinations

Each fetus was sexed, weighed, and examined for external abnormalities. Live fetuses were sacrificed via injection with an appropriate barbiturate followed by exsanguination.

Approximately one-half of all fetuses from each litter were selected by computer and processed for visceral examination. Heads were removed, stored frozen on dry ice, and cross-sectioned using the Wilson's sectioning technique (Astroff et al., 2002). Internal organs of the thoracic and abdominal cavities were examined in the fresh state using Staples' technique (Stuckhardt and Poppe, 1984). Fetuses identified for modified Staples' examination (visceral examination) were then discarded.

Using the Alizarin Red S staining method (Dawson, 1926), remaining fetuses were eviscerated and processed for skeletal evaluation, which included an examination of the skull, vertebral column, rib cage, pectoral and pelvic girdles, long bones, and extremities. Bone alignment and degree of ossification were assessed. Fetuses processed for skeletal evaluation were retained in glycerin, with thymol added as a preservative.

Findings were classified as variations or malformations. Malformations are developmental deviations which are macroscopic structural changes, incompatible with life, or may affect the quality of life. Variations are structural deviations thought to have no effect on body conformity or the well-being of an animal.

2.6. Statistical and data analysis

Except where otherwise stated, tests were performed using a two-sided risk at the 5% level of significance. Tables supporting statistical

Table 1
Study design for toxicity animals and toxicokinetic animals.

Group[a]	Number of Mated Females	Dose Level (mg/kg/day)	Dose Concentration[b,c] (mg/mL)	Dosing Schedule
Toxicity Animals				
1 (Control)	22	0	0	GD 6 through 17
2 (Low)	22	9	0.9	GD 6 through 17
3 (Mid)	22	90	9.0	GD 6 through 17
4 (High)	22	225	22.5	GD 6 through 17
Toxicokinetic Animals				
1 (Control)	3	0	0	GD 6 through 17
2 (Low)	6	9	0.9	GD 6 through 17
3 (Mid)	6	90	9.0	GD 6 through 17
4 (High)	6	225	22.5	GD 6 through 17

GD = Gestation Day.
[a] Group 1 (toxicity or toxicokinetic animals) were administered vehicle control article only.
[b] Animals were dosed at a dose volume of 10 mL/kg.
[c] Concentrations were within ±10% of nominal.

Table 2
Toxicokinetic analyses study design.

Group	Set	Phase Day	Time Points[a]
1	Three TK animals/ group/time point	GD 6 and 17	Predose and 0.25 h post-dose
2, 3, 4	Three TK animals/ group/time point	GD 6 and 17	Predose and 0.25, 0.5, 1, 3, and 6 h post-dose[b]

GD = Gestation Day; TK = Toxicokinetic.
[a] Blood collection times were approximate.
[b] The plasma half-life of the agent is about 30 min, so 6 h post-dose should encompass entire profile.

Table 3
Toxicokinetic parameters.

Parameter	Description
C_0	Back-extrapolated concentration at time 0.
C_{max}	Maximum observed concentration.
DN C_{max}	Dose normalized maximum concentration, calculated as C_{max}/dose.
T_{max}	Time of maximum observed concentration.
$AUC_{0\text{-}t}$	Area under the curve from time 0 to the time of the last measurable concentration, calculated using the linear trapezoidal rule.
$AUC_{0\text{-}6}$	Area under the curve from time 0 to hour 6, calculated using the linear trapezoidal rule.
DN $AUC_{0\text{-}6}$	Dose normalized $AUC_{0\text{-}6}$, calculated as $AUC_{0\text{-}6}$/dose.
$AUC_{0\text{-}inf}$	Area under the curve from time 0 to infinity (GD 6 only), calculated as $AUC_{0\text{-}inf} = AUC_{0\text{-}t} + C_t/\lambda_z$, where C_t is the last observed quantifiable concentration and λ_z is the elimination rate constant.
$t_{1/2}$	Elimination half-life, calculated as $\ln(2)/\lambda_z$.
CL	Clearance, calculated as Dose/$AUC_{0\text{-}inf}$ on GD 6 and Dose/$AUC_{0\text{-}tau}$ on GD 17
V_{ss}	Volume of distribution at steady-state, calculated as CL * $MRT_{0\text{-}inf}$
AR	Accumulation ratio, calculated as (GD 17 C_{max} or $AUC_{0\text{-}6}$)/(GD 6 C_{max} or $AUC_{0\text{-}6}$)

evaluations were generated using the Pristima preclinical software and or Tox Reporting.

For food consumption, body weight, and body weight change data, Levene's test was performed to test for equality of variances between groups. Where Levene's test was significant ($p < 0.05$), a rank transformation (to stabilize the variances) was applied to the data prior to analysis. Where data for only 2 groups were available for analysis, data were analyzed using a two-sample *t*-test. Where there were more than two groups available for analysis, data was analyzed using ANOVA. Where the group effect from the ANOVA was not significant ($p > 0.05$), no further analyses was performed. Where the group effect from the ANOVA was significant ($p < 0.05$) pairwise comparisons were performed. For comparisons against a single or combined identical controls, pairwise comparisons were performed using Dunnett's test. For comparisons not against a single or combined identical controls, pairwise comparisons were performed using t-tests. All pairwise comparisons were evaluated at the 5.0% two-tailed probability levels. Data from non-pregnant animals were excluded from summary calculations.

For uterine weight (including carcass weights) Levene's test was conducted to test for equality of variances between groups. Where Levene's test was not significant ($P > 0.05$), a statistical comparison across test article-treated and control groups was conducted using a one-way analysis of variance (ANOVA). If the group effect of the ANOVA was not significant ($P > 0.05$), no further analyses was conducted. If the ANOVA was significant ($P \leq 0.05$), Dunnett's test was used for pairwise comparisons between each test article-treated group and the control group. If Levene's test was significant ($P \leq 0.05$), a Kruskal-Wallis nonparametric ANOVA was conducted. If the Kruskal-Wallis test was significant ($P \leq 0.05$), pairwise comparisons of each treated group with the control group were made using the Wilcoxon Rank Sum Test. Data from non-pregnant animals were excluded from summary calculations.

Cesarean section data (excluding uterine and carcass weights), percent male data, and fetal defect data (mean litter percent) were evaluated using a Kruskal-Wallis nonparametric ANOVA and the Wilcoxon Rank Sum Test, as described previously. Fetal defect data (proportion of litters affected) were evaluated using Fisher's Exact Test (one-sided, increasing).

Mean live fetal weights (males, females, and combined sexes) were analyzed using analysis of covariance (ANCOVA). The litter size (live and dead fetuses) was used as the covariate. Where the group effect from the ANCOVA was significant ($P \leq 0.05$), Dunnett's test was used for pairwise comparisons between each test article-treated group and the control group.

Toxicokinetic statistical analysis was limited to the calculation of means and standard deviations.

3. Results

3.1. Clinical observations

Non-adverse test article-related clinical observation of discolored bedding (yellow or orange) was noted for all MB-102-treated groups. Observations of discolored skin (ears, tail, or whole body; yellow or orange), discolored eyes (yellow or orange), and/or discolored pelage (yellow or orange) were noted post-dose for animals administered 90 or 225 mg/kg/day. Based on the nature of the findings (yellow/orange discoloration) and the color and fluorescent properties of the test article, these observations for all MB-102-treated groups were considered test article related but nonadverse.

3.2. Toxicokinetics

Toxicokinetic results are presented in Table 4. Exposure, as assessed

Table 4
Summary of the MB-102 toxicokinetic parameters in the plasma of pregnant rats.

GD	Dose Group	Dose Level (mg/kg/day)	C_0[a] (μM)	C_{max} (μM)	DN C_{max} [(μM)/(mg/kg/day)]	T_{max} (h)	AUC_{0-6} (h*μM)	DN AUC_{0-6} [(h*μM)/(mg/kg/day)]	$t_{1/2}$ (h)	AR C_{max}	AR AUC_{0-6}
6	2	9	100	55.3	6.15	0.250	52.4	5.82	0.393	NA	NA
	3	90	918	553	6.14	0.250	522	5.80	0.405	NA	NA
	4	225	2780	1460	6.47	0.250	1350	5.98	0.538	NA	NA
17	2	9	128	65.9	7.33	0.250	62.2	6.91	0.407	1.19	1.19
	3	90	1230	701	7.79	0.250	699	7.77	0.605	1.27	1.34
	4	225	2760	1590	7.04	0.250	1640	7.29	0.607	1.09	1.22

NA = Not applicable.
[a] C_0 results were calculated with between 30 and 40% back extrapolation in all instances. As a result, C_0 results should be interpreted cautiously.

by MB-102 C_{max} and AUC_{0-6}, increased with the increase in dose level from 9 to 225 mg/kg/day. The increases in C_{max} and AUC_{0-6} values were generally dose proportional. No accumulation of MB-102 was observed after multiple doses in pregnant rats. Based on these data, the no observed adverse-effect level (NOAEL) for maternal and fetal toxicity was 225 mg/kg/day (GD 6 C_{max} and AUC_{0-6} values of 1460 μM and 1350 h*μM, respectively; GD 17 C_{max} and AUC0-6 values of 1590 μM and 1640 h*μM, respectively).

3.3. Body weight

Body weight data are depicted graphically in Fig. 2 and summarized in Table 5. No test article-related effect on mean body weight or mean body weight gain was observed for any dose level. Mean body weight gain was statistically significantly increased from GD 12 through 13 for animals administered 225 mg/kg/day, compared with controls. During this interval, mean body weight gain for animals administered 225 mg/kg/day was increased by 155% (3.1 g), compared with controls; however, this increase was attributable to weight gains noted for all animals, except one, administered 225 mg/kg/day, compared with weight gains for only 14 of 22 control animals. Despite the significance of this interval, the increase was transient and all other mean body weight intervals were comparable with controls.

3.4. Food consumption

Food consumption data are depicted graphically in Fig. 3 and summarized in Table 6. No test article-related effect on mean food consumption was observed for any dose level.

Table 5
Summary of body weight gain.

Group		GE: 4-6	GE: 8-9	GE: 12-13	GE: 15-16	GE: 18-21	GE: 6-21
1	n	22	22	22	22	22	22
	Means	21.1	6.9	2.0	11.1	22.1	83.5
	SDVs	3.14	5.81	4.53	4.6	10.57	16.58
2	n	21	21	21	21	21	21
	Means	19.0	4.1	2.0	9.9	18.8	77.2
	SDVs	2.66	4.13	4.39	4.22	8.69	18.06
3	n	22	22	22	22	22	22
	Means	20.3	6.2	3.2	7.7	22.1	82.1
	SDVs	4.12	6.26	3.74	3.67	14.82	15.75
4	n	22	22	22	22	22	22
	Means	19.1	5.5	5.1 [a]H	10.3	26.3	90.3
	SDVs	3.22	5.7	3.02	2.68	8.08	13.87

GE = Gestation.
See Table 7 for accounting of n.
[a] H = Dunnett Exact Homogenous Test Significance at 0.05 Level.

Mean food consumption was statistically significantly reduced by 8.1% (6.4 g) from GD 15 through 18 for animals administered 9 mg/kg/day, compared with controls; however, this significant reduction was transient and not dose responsive. All other intervals were comparable

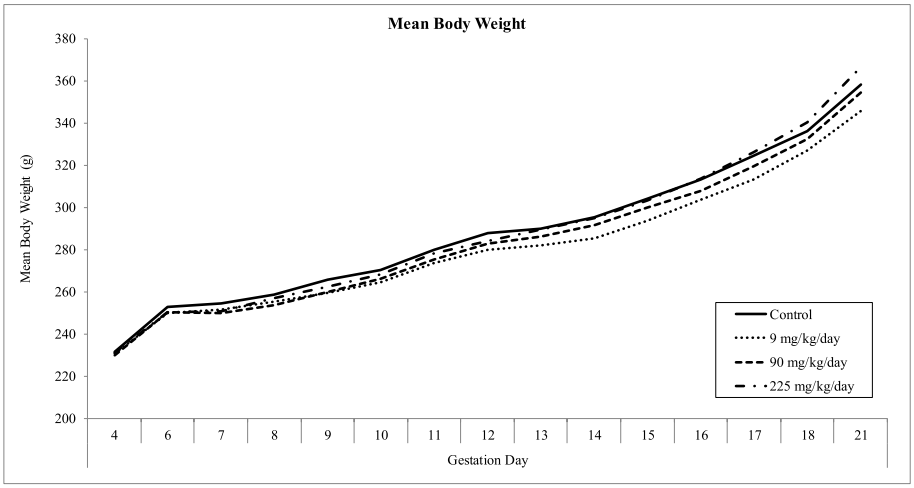

Fig. 2. Mean body weight.

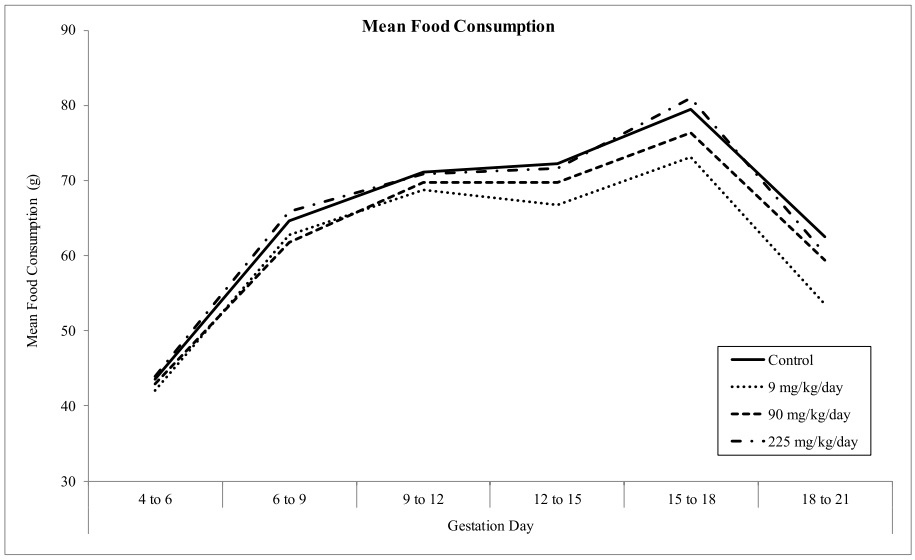

Fig. 3. Mean food consumption.

Table 6
Summary of mean food consumption.

Group		GE: 4-6	GE: 6-9	GE:9-12	GE:12-15	GE:15-18	GE: 18-21
1	n	22	22	22	21	22	22
	Means	43.6	64.4	71.1	72.2	79.5	62.5
	SDVs	4.82	6.57	6.05	8.28	9.3	11.3
2	n	21	21	21	21	21	21
	Means	42.0	62.8	68.8	66.8	73.1*r	53.5
	SDVs	4.89	5.55	5.24	5.03	5.45	9.67
3	n	22	22	22	22	22	22
	Means	42.9	61.7	69.7	69.7	76.4	59.4
	SDVs	4.15	6.97	7.45	8.16	7.86	12.96
4	n	22	22	22	22	22	22
	Means	43.9	65.9	70.9	71.6	81.0	60.3
	SDVs	4.19	5.99	5.67	5.23	5.33	4.16

GE = Gestation.
*r = Wilcoxon Rank Sum Test Significance at 0.05 Level.
See Table 7 for accounting of n.

with control.

3.5. Reproductive performance

The pregnancy rate was 100, 95, 100, and 100% for animals administered vehicle control article or 9, 90, or 225 mg/kg/day, respectively. Summary of the reproductive performance is shown in Table 7.

Table 7
Summary of reproductive performance.

Treatment Group	Control	9 mg/kg	90 mg/kg	225 mg/kg
Total Females	22	22	22	22
Pregnant Females	22	21	22	22
Non Pregnant Females	0	1	0	0
Pregnant Females w/Total Litter Loss	0	1	0	0
Females with Live Fetuses	22	20	22	22

3.6. Caesarean section analyses (maternal necropsy)

No test article-related effect on cesarean section parameters was observed at any dose level. All parameters were comparable to controls. Table 8 summarizes the Cesarean Section parameter data.

3.7. Summary of fetal evaluations noted

As previously stated, variations are structural deviations thought to have no effect on body conformity or the well-being of an animal. All observed external anomalies were limited to one fetus from an animal administered vehicle control article, one fetus from an animal administered 90 mg/kg/day, and three fetuses from an animal administered 225 mg/kg/day. No test article-related fetal external abnormalities were observed for any MB-102 dose level.

The single fetus from the animal administered 90 mg/kg/day was observed with malformations of omphalocele, hyperflexion, cleft palate, brachydactyly, and syndactyly, and variations of edema and curled tail. The single fetus from the animal administered vehicle control article was also observed with the malformation of omphalocele. The fetuses, (n = 3), from the animal dosed at 225 mg/kg/day were observed with malformations of gastroschisis, exencephaly, small or absent eye bulge, hyperflexion, cleft palate, and protruding tongue, and/or the variation of curled tail.

These fetal external anomalies were considered not test article-related based on the limited occurrence of these anomalies to single litters in the groups administered 90 or 225 mg/kg/day and similar incidences in the control group, as well as the lack of effect on Cesarean section parameters.

No test article-related fetal visceral abnormalities were observed for any MB-102 dose level.

The visceral malformation of persistent ductus arteriosus was noted for one fetus from one animal administered 225 mg/kg/day. This malformation was of single occurrence and within the Covance historical control database of the testing facility, and was considered not test article-related.

The noted visceral variations were single incidence within a group, noted in controls only, lacked dose responsiveness, and/or were within the same historical control database, and they too were considered not test article-related.

Table 8
Summary of cesarean section results.

# of Pregnant Females	Group	Control	9 mg/kg	90 mg/kg	225 mg/kg
	(n)	22	21	22	22
Corpora Leuta	(n)	22	21	22	22
	Mean	13.2	13.9	13.8	13.9
	SD	2.87	2.48	1.71	2.08
Implantation Sites	(n)	22	21	22	22
	Mean	11.3	12.2	12.0	12.8
	SD	3.43	2.75	2.32	2.06
Preimplantation Loss (%)	(n)	22	21	22	22
	Mean	15.78	11.6	13.66	7.22
	SD	19.59	16.72	11.89	9.81
Total Resorptions	(n)	22	21	22	22
	Mean	0.8	0.5	0.6	0.7
	SD	1.44	1.08	0.85	1.70
Postimplantation Loss (%)	(n)	22	21	22	22
	Mean	7.74	7.63	5.70	6.02
	SD	16.35	22.20	9.90	15.47
Live Fetuses	(n)	22	21	22	22
	Mean	10.5	11.7	11.4	12.1
	SD	3.69	3.21	2.63	2.98
Dead Fetuses	(n)	22	21	22	22
	Mean	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0
# Female Fetuses/Litter	(n)	22	21	22	22
	Mean	4.8	6.0	5.8	6.4
	SD	1.95	2.45	2.13	1.99
# Male Fetuses/Litter	(n)	22	21	22	22
	Mean	5.7	5.7	5.5	5.8
	SD	2.55	2.22	2.06	2.20
Mean Fetal Wt. (g)	(n)	22	20	22	22
	Mean	5.98	5.60	5.64	5.78
	SD	0.57	0.32	0.43	0.48
Mean Fetal Wt. (Females)	(n)	21	20	22	22
	Mean	5.79	5.48	5.50	5.65
	SD	0.54	0.32	0.48	0.58
Mean Fetal Wt. (Males)	(n)	22	20	22	22
	Mean	6.11	5.72	5.83	5.96
	SD	0.56	0.33	0.45	0.44
Gravid Uterine Wt.	(n)	22	20	22	22
	Mean	83.4	87.77	87.28	92.16
	SD	26.05	24.13	18.53	23.30

See Table 7 for accounting of n.

3.8. Summary of skeletal evaluations

No test article-related fetal skeletal abnormalities were observed for any MB-102 dose level.

All noted skeletal malformations were limited to a single fetus from a dam administered 90 mg/kg/day and two litters from dams administered 225 mg/kg/day, one of which was also noted with multiple external malformations.

One animal administered 225 mg/kg/day was observed with three severely externally malformed fetuses. These externally malformed fetuses were also observed upon skeletal examination with one or more of the following: misshapen scapula, basisphenoid, frontal, interparietal, lacrimal, parietal, presphenoid, squamosal, tympanic annulus, and/or zygomatic arch; fused frontal/parietal; absent interparietal; short maxilla; small premaxilla; absent presphenoid; absent supraoccipital; fused sternebra; fused cervical arch; fused ribs; and/or fused thoracic arch. Another animal administered 225 mg/kg/day had two fetuses noted with absent lumbar vertebrae. The fetus from animal administered 90 mg/kg/day was observed with a malpositioned phalanx.

Aside from the single malformation in the mid-dose group, these malformations were limited to two high-dose litters. The malformations in these litters were not physiologically-related (cervical, craniofacial, and thoracic malformations in one animal) versus lumbar in a second animal. Nor were the fetuses from a third animal identified with external abnormalities. These malformations showed no embryological relation and were limited to a single litter. As such, they were considered not test article-related.

The noted skeletal variations were single incidence within a group, noted in controls, lacked dose responsiveness, noted in the three extremely malformed fetuses from a single animal, and/or were within the historical control database previously mentioned, and were considered not test article-related.

4. Discussion and conclusions

Previously reported safety and toxicity studies of MB-102 demonstrated negligible safety concerns and were critical to the FDA clearance of this agent to advance to Phase II human clinical trials. The nonclinical studies included herein further supplement the overall safety and toxicity profile of this novel fluorescent tracer agent. The focus of these studies was specifically designed to assess maternal toxicity and potential effects on embryo-fetal development in pregnant rats following the intravenous administration of MB-102. Included in this series of studies were mortality, food consumption, body weight gain, reproductive indices, Cesarean section parameters, fetal mortality and overall clinical observations. A toxicokinetic profile of this agent was also determined. All animals survived to the conclusion of the study. With regard to food consumption and body weight gain, there were no significant differences between the untreated control group and the three groups of animals dosed with MB-102. Clinical observations were limited to discoloration of the skin, eyes and/or pelage at the two higher doses administered. These findings are consistent with this fluorescent tracer agent (Shieh et al., 2020) and were considered non-adverse. (Note that in the human clinical studies, the dose is such that the test article coloration is never observed.) No test article-related fetal external abnormalities were observed at any MB-102 dose level, and no test article-related fetal skeletal abnormalities were observed at any MB-102 dose level either. All observed external anomalies were limited to one fetus from an animal administered vehicle control article, one fetus from an animal administered 90 mg/kg/day, and three fetuses from an animal administered 225 mg/kg/day.

Exposure, as assessed by MB-102 C_{max} and $AUC_{0\text{-}6}$, increased with the increase in dose level from 9 to 225 mg/kg/day. The increases in C_{max} and $AUC_{0\text{-}6}$ values were generally dose proportional. No accumulation of MB-102 was observed after multiple doses in pregnant rats. Based on these data, the no observed adverse-effect level (NOAEL) for

maternal and fetal toxicity was 225 mg/kg/day.

The study reported herein was performed in accordance with the United States Food and Drug Administration (FDA) Good Laboratory Practice (GLP) Regulations, Title 21 of the United States Code of Federal Regulations Part 58 (except for body weights and observations from animal supplier prior to dosing).

In conclusion, intravenous administration of MB-102 was not associated with any test article related effects on mortality, food consumption, body weight gain, Cesarean section parameters, macroscopic observations, reproductive performance, or developmental toxicity including teratogenic potential in pregnant rats.

The final pivotal clinical study for this GFR methodology is expected to commence in the near-term.

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CRediT authorship contribution statement

Joseph E. Bugaj: Data curation, Writing – original draft, Validation. **Richard B. Dorshow:** Conceptualization, Resources, Writing – review & editing, Project administration.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: RBD is a stock owner and employee of MediBeacon Inc. JEB is a paid consultant of MediBeacon Inc.

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