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TADAFER II: Tadalafil treatment for fetal growth restriction - a study protocol for a multicenter randomized controlled phase II trial.

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1 **Title: TADAFER II: Tadalafil treatment for fetal growth restriction - a study protocol for**
2 **a multicenter randomized controlled phase II trial.**

3
4 Running head: Tadalafil for fetal growth restriction

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29 **Disclosure**

30 The authors declare no conflict of interest.

31
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1 **Title: TADAFER II: Tadalafil treatment for fetal growth restriction - a study protocol for**
2 **a multicenter randomized controlled phase II trial.**

3
4 **ABSTRACT**

5 **Introduction:** There is no proven therapy to reverse or ameliorate fetal growth restriction
6 (FGR). Sildenafil, a selective phosphodiesterase 5 (PDE5) inhibitor, has been reported to
7 potentially have a therapeutic role in FGR, but this has not been established. Tadalafil is also a
8 selective PDE5 inhibitor and has a longer half-life and a more rapid onset of action than
9 sildenafil. We have demonstrated efficacy for tadalafil on fetal growth in FGR and the
10 short-term outcomes and feasibility of tadalafil in FGR. Based on the hypothesis that tadalafil
11 will safely increase the likelihood of increased fetal growth in FGR, we have designed this
12 phase II study to prospectively evaluate the efficacy and safety of tadalafil in FGR.

13 **Methods and analysis:** This study is a multicenter randomized controlled phase II trial. A total
14 of 140 fetuses with FGR will be enrolled from major medical centers in Japan. Fetuses will be
15 randomized to receive either the conventional management for FGR, according to the guidelines
16 in Japan, or a once daily treatment with 20 mg of tadalafil along with the conventional
17 management, until delivery. Fetal growth velocity from the first day of the treatment to birth has
18 been defined as the primary endpoint. To minimize bias in terms of fetal baseline conditions and
19 timing of delivery, a fetal indication for delivery is established in this study based on the results
20 from a Japanese multicenter survey. The investigator will evaluate fetal baseline conditions at
21 enrollment and will decide the timing of delivery based on this fetal indication. Infants will be
22 followed up for development until 1.5 years of age.

23 **Ethics and dissemination:** This study was approved by the Institutional Review Board of Mie
24 University Hospital and each participating institution. Our findings will be widely disseminated
25 through peer-reviewed publications.

26 **Trial registration:** UMIN Clinical Trials Registry UMIN000023778.
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1 **Strengths and limitations of this study**

- 2 • This is a multicenter randomized controlled phase II trial to prospectively evaluate the
3 efficacy and safety of tadalafil treatment in fetuses with fetal growth restriction (FGR), for
4 which there is no proven therapy.
- 5 • This trial will include the participation of major medical centers providing treatment for
6 fetuses with FGR according to the guidelines for obstetrical practice in Japan.
- 7 • To minimize bias in terms of fetal baseline conditions and timing of delivery, a fetal
8 indication for delivery is established in this study on the basis of the results from a
9 multicenter survey in Japan.
- 10 • The possible limitation is related to open-label trial features, in which enrolled participants
11 receive either the conventional management for FGR according to the guidelines for
12 obstetrical practice in Japan, or a once daily treatment with 20 mg of tadalafil added to the
13 conventional management.

1 INTRODUCTION

2 Neonatal intensive care has improved over the past few decades, and morbidity among
3 infants, including those who are premature, continues to decline. Premature infants with
4 intrauterine growth restriction, however, still have high mortality and morbidity. The multicenter
5 survey[1] of very low birth weight (VLBW) infants in Japan conducted by Kusuda and Ikeda *et*
6 *al.* revealed that mortality in neonatal intensive care units (NICU), of small gestational age
7 (SGA) infants born before 30 weeks gestation, was significantly higher than that of appropriate
8 for gestational age (AGA) infants (unpublished data). To prevent fetal growth restriction (FGR),
9 nutritional and dietary supplementation, bed rest, and aspirin therapy have been investigated,
10 but there is insufficient evidence for the routine indication of any of these treatments.[2] There
11 is also no proven therapy to reverse or ameliorate established FGR.[3]

12 Increases in uteroplacental blood flow during pregnancy via angiogenesis and
13 vasodilation contribute to adequate fetal growth. Vasodilation in the uteroplacental unit is
14 considered to be due to the production and local release of nitric oxide (NO), which stimulates
15 cyclic guanosine monophosphate (cGMP) production.[4] cGMP is inactivated mainly by
16 phosphodiesterases (PDE), and the predominant PDE isoform present in the vascular smooth
17 muscle is PDE5. Because inhibitors of PDE5, which is a cGMP-specific PDE, exert their
18 pharmacological action by dilating arteries and increasing blood flow, as proven in erectile
19 dysfunction and pulmonary hypertension, recent studies have suggested a potential therapeutic
20 role for PDE5 inhibitors in treating FGR.[5] Sildenafil, a selective PDE5 inhibitor, has been
21 shown to improve endothelial function in myometrial small arteries removed from women with
22 pre-eclampsia and FGR.[6, 7] However, although sildenafil has been reported to affect maternal
23 hypertension, it has not been shown to affect FGR in studies in FGR model rats induced by
24 L-NG-nitroarginine methyl ester (L-NAME) treatment. In these studies, FGR was not affected
25 by sildenafil except in one report, by Baijnath *et al.*[8-11] Baijnath *et al.* demonstrated that
26 L-NAME-induced FGR was improved by sildenafil treatment from 4 days postcoitum (d.p.c.) to
27 8 d.p.c. but not from 8 d.p.c. to 14 d.p.c.[10] Chorioallantoic attachment occurs at 8 d.p.c., and
28 the mature circulatory pattern of maternal blood through the placenta is established by 10 d.p.c.
29 in mouse placenta.[12, 13] In considering the development of fetoplacental circulation in
30 rodents, the effect of sildenafil on fetal growth associated with placental blood flow via an
31 NO-dependent pathway was not manifested. In a clinical study, it was reported that sildenafil
32 was associated with increased fetal abdominal circumference (AC) growth velocity in severe

1 early-onset FGR, but the authors did not report on fetal growth velocity and birth weight.[14]
2 Recently, the STRIDER UK group has found no evidence of a beneficial effect of sildenafil on
3 survival or short-term neonatal outcomes.[15]

4 Tadalafil is also a selective PDE5 inhibitor and has a longer half-life and a more rapid
5 onset of action than sildenafil.[5] Tadalafil has been used to treat pulmonary hypertension in
6 pregnant women and the Food and Drug Administration in the United States has rated tadalafil
7 as pregnancy category B.[16] When taking sildenafil with a high-fat meal, the time to maximum
8 plasma concentration increases and the peak plasma concentration falls.[17] In contrast, Forgue
9 *et al.* reported that food intake had a negligible effect on the bioavailability of tadalafil, and also
10 reported that there was no clinically meaningful effect of gender on tadalafil
11 pharmacokinetics.[18] Our animal experiments demonstrated that tadalafil treatment dilates the
12 maternal blood sinuses in the placenta, which leads to increased placental growth factor (PlGF)
13 production, and contributes to facilitating fetal growth.[19] Because tadalafil treatment was
14 started after blood spaces in the placenta were narrowed by L-NAME treatment and elevated
15 urinary excretion of cGMP in these animal experiments, we can safely presume that tadalafil
16 treatment contributes to facilitating fetal growth in the context of the mechanisms associated
17 with NO signaling. In addition, we retrospectively analyzed 11 Japanese singleton pregnant
18 women with FGR who received tadalafil along with conventional management for FGR at Mie
19 University Hospital from July 2015 to February 2016 (tadalafil group).[20] These women were
20 matched for maternal age, parity, gestational age (GA), and estimated fetal weight at enrollment
21 with 14 singleton pregnant women who had received only the conventional management for
22 FGR in 2014 (conventional management group). The conventional management for FGR was
23 performed according to the guidelines for obstetric practice in Japan.[21] This retrospective
24 study showed that both fetal growth velocity from enrollment to birth and birth weight were
25 significantly higher in the tadalafil group than in the conventional management group. Moreover,
26 the prevalence of respiratory distress syndrome (RDS) was significantly lower in the tadalafil
27 group than in the conventional management group. After the retrospective study, we conducted
28 a phase I clinical trial to ensure the safety of tadalafil treatment for FGR.[22] There were no
29 serious maternal adverse events for daily tadalafil doses of 10 mg, 20 mg, and 40 mg. More
30 patients who were administered 40 mg tadalafil daily experienced mild adverse events than
31 those administered 10 mg or 20 mg tadalafil daily. In regards to fetal adverse events,
32 intrauterine fetal death occurred in one case. In this case, the pregnant woman was prescribed 40

1 mg tadalafil daily and fetal growth had been progressing at a rate of 22 g/day. At 36 weeks gestation, fetal movement suddenly ceased and a diagnosis of intrauterine fetal death was made. Thereafter, the fetus was delivered vaginally, and velamentous insertion of the umbilical cord was identified. Immediately, the safety evaluation committee investigated the incident's relationship to tadalafil. This committee analyzed the case and concluded that the intrauterine fetal death was due to velamentous insertion of the umbilical cord.[23] We concluded that tadalafil treatment was feasible in pregnant women with FGR.[22]

Based on the above, we have hypothesized that tadalafil therapy will safely increase the likelihood of increased fetal growth in fetuses with FGR and have designed this multicenter randomized controlled phase II study to establish evidence for fetal therapy with tadalafil. This study, funded by the Japan Agency for Medical Research and Development (AMED), will prospectively evaluate the safety and efficacy of tadalafil in FGR with the participation of major medical centers providing treatment for fetuses with FGR according to the guidelines for obstetrical practice in Japan.

METHODS

Study design

This study is a multicenter randomized controlled phase II trial.

Study period

The planned study period is from the date of ethics approval to February 2021. The Patient Registration Period will last until December 2018. The children's outcome will be followed up for 1.5 years after birth. Data collected by the end of the Neonatal Evaluation Period will be subjected to statistical analysis.

Patient Registration Period: date of ethics approval to December 2018.

Children's Outcome Follow-up Period: 1.5 years after the last birth.

Patient selection

Inclusion criteria are as follows: (1) Pregnant women ≥ 20 years; (2) Estimated fetal weight (EFW) less than 1.5 standard deviations of the mean EFW for GA; (3) GA between 20 + 0 and 33 + 6 weeks; (4) The expected date of confinement is determined using the criteria of the guidelines for obstetrical practice in Japan (2014); (5) Singleton pregnancy; and (6) Signed written informed consent.

Exclusion criteria are as follows; (1) A result from the antepartum fetal tests, done at enrollment, which indicates that delivery should be attempted*; (2) A history of allergy to

1 1 tadalafil; (3) Concurrent medications that interact adversely with tadalafil; (4) Contraindication
 2 2 of tadalafil treatment due to renal disease; (5) Contraindication of tadalafil treatment due to liver
 3 3 disease; (6) Contraindication of tadalafil treatment due to uncontrolled arrhythmia, hypertension
 4 4 (BP >170/100 mmHg), and hypotension (BP <80/40 mmHg); (7) Fetus with suspected
 5 5 chromosomal disorder and/or multiple congenital anomalies; (8) Contraindication of tadalafil
 6 6 treatment due to retinitis pigmentosa, coagulation defect, active gastric and/or intestinal ulcer, or
 7 7 venous obstructive disease; and (9) The investigator decides that entry is inappropriate.

8 * To minimize bias in terms of fetal baseline condition at enrollment, a fetal indication for
 9 delivery is established on the basis of the results from the multicenter survey of VLBW infants
 10 in Japan using a network database, in which the 82 level III perinatal centers were registered.
 11 The survey data included infant survival rate in the NICU, categorized by birth weight and
 12 gestational week at birth (Figure 1).[1] The infant survival rate data acquired from the survey
 13 were preprocessed with the moving average method and divided into three groups. The first
 14 group was defined as “Zone 1” where the infant survival rate in the NICU was less than 60%.
 15 The second group was defined as “Zone 2” where the infant survival rate in the NICU ranged
 16 from 60 to 95%. The third group was defined as “Zone 3” where the infant survival rate in the
 17 NICU was 95% or higher. All patients in our study will undergo antepartum fetal tests
 18 consisting of the evaluation of fetal well-being by ultrasonography, including Doppler imaging
 19 of umbilical arterial blood flow, non-stress test, contraction stress test, and biophysical profile
 20 scoring depending on GA, to evaluate possible pregnancy termination by the investigator at
 21 enrollment (Table 1). [21, 23, 24]

22 **Table 1. A fetal indication for delivery in the TADAFER II study.** [21, 23, 24]

Infant survival rate in the NICU (See Figure 1)	
Zone 1	Decide timing of delivery depending on available therapeutic measures at the NICU in each institute.
Zone 2	Consider delivery if at least one of three findings is made, but place give high priority on the determination by the investigators. 1. Reversed umbilical artery blood flow during diastole. 2. Score less than 4 on the fetal biophysical profile score. 3. Feat heart rate patterns in the orange or red category for more than 30 minutes. [24]
Zone 3	Consider delivery if at least one of five findings is made, but place give high priority on the determination by the investigators. 1. Reversed or absent umbilical artery blood flow during diastole. 2. Score less than 4 on the fetal biophysical profile score. (Score less than 6 on the fetal biophysical profile score if oligohydramnios is present.) 3. Feat heart rate patterns in the orange or red category for more than 30 minutes. [24] 4. Positive contraction stress test. 5. Impaired fetal head circumference growth for more than 2 weeks.

23

1 **Registration**

2 The study protocol defines all of the procedures and schedules that the investigator must
3 abide by to complete this clinical study, including patient selection and registration, fetal
4 treatment of FGR, and follow-up (Figure 2). Patients that satisfy all inclusion criteria and do not
5 meet any of the exclusion criteria will be eligible for inclusion in the study. Individual study
6 sites will be responsible for guiding potential participants through the informed consent process,
7 including patients who have been referred to them for treatment purposes. The investigator will
8 enter an eligible patient's information into the Eligibility Confirmation Form on the website of
9 this clinical trial (the Clinical Trial Data Management System:
10 <http://scope.mie-cts.net/rd/p01.php> Japanese-only website). The data management system will
11 check the contents of the form before registering the patient. For patients who meet all inclusion
12 criteria without violating any of the exclusion criteria listed above, the data management system
13 will register and allocate them to the two arms in an allocation ratio of 1:1, one group receiving
14 the conventional management of FGR according to the guidelines for obstetrical practice in
15 Japan,[21] and the other receiving once-daily treatment with 20 mg tadalafil added to
16 conventional management after adjustment for study sites and GA (<28 or ≥28 weeks of
17 gestation). The investigators are blinded to the allocation algorithm. Enrolled participants will
18 receive fetal therapy within 7 days of registration. The investigator will enter the patients' data
19 into the Case Report Form on the website of this clinical trial (the Clinical Trial Data
20 Management System: <http://scope.mie-cts.net/rd/p01.php>).

22 **Fetal Treatment Protocol**

23 The investigator will provide the fetal therapy as described below.

24 *Arm A:* the conventional management of FGR according to the guidelines for obstetrical
25 practice in Japan.[21] Briefly, the conventional management of FGR consists of evaluation of
26 fetal well-being on ultrasonography, including Doppler imaging of umbilical arterial blood flow,
27 non-stress test, contraction stress test, and biophysical profile scoring depending on GA to
28 evaluate possible pregnancy termination.

29 *Arm B:* once-daily treatment with 20 mg tadalafil added to the conventional management until
30 delivery.

31 The investigators are blinded to the allocation algorithm. Enrolled participants will receive fetal
32 therapy within 7 days of registration.

34 **Endpoints**

35 **(1) Primary endpoint**

36 Fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day).

The primary endpoint is fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day), and is calculated using the following formula:

$$\text{Fetal growth velocity (g/day)} = \frac{\text{Birthweight} - \text{EFW at the first day of the treatment [g]}}{\text{Days of the treatment [days]}}$$

Rationale for the primary endpoint

Our primary hypothesis is that tadalafil therapy will increase the likelihood of increased fetal growth velocity in fetuses with FGR. Taking into account the results of our retrospective study demonstrating that tadalafil treatment increased fetal growth velocity (g/day), which was the primary outcome of the retrospective study, and decreased the incidence of RDS, an improvement in fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day) is an important indicator of the therapeutic benefits for fetuses with FGR.[20]

(2) Secondary endpoints

1) Completion rate of the treatment regimen.

Completion rate of the treatment regimen is defined as the percentage of enrolled patients who receive the protocol-defined treatment for more than 7 days.

2) Efficacy endpoints.

i) Estimated fetal weight (g).

Estimated fetal weight (EFW) is calculated using the following formula:[25]

$$\text{EFW (g)} = 1.07 \times (\text{biparietal diameter: BPD})^3 + 0.3 \times (\text{abdominal circumference: AC})^2 \times (\text{femur length: FL})$$

ii) Fetal growth velocity in the two weeks after the protocol-defined treatment and in the two weeks after one week of the protocol-defined treatment (g/day).

Fetal growth velocity in the two weeks after the protocol-defined treatment (g/day) is calculated using the following formula:

$$\text{Fetal growth velocity in the two weeks after the protocol – defined treatment (g/day)} = \frac{(\text{EFW two weeks after the treatment} - \text{EFW at the first day of the treatment [g]})}{14 \text{ [days]}}$$

and fetal growth velocity in the two weeks after one week of the protocol-defined treatment (g/day) is calculated using the following formula:

$$\text{Fetal growth velocity in the two weeks after one week of the treatment (g/day)} = \frac{(\text{EFW three weeks after the treatment} - \text{EFW one week after the treatment [g]})}{14 \text{ [days]}}$$

iii) Fetal growth rate in the two weeks after the protocol-defined treatment and from the first day of the protocol-defined treatment to birth (%/day).

1 Fetal growth rate in the two weeks after the protocol-defined treatment (%/day) is calculated
2 using the following formula:

$$3 \quad \text{Fetal growth rate in the two weeks after the protocol-defined treatment (\%/day)} \\ 4 = \frac{\text{EFW two weeks after the treatment} - \text{EFW at the first day of the treatment [g]} \times 100}{\text{EFW at the first day of the treatment [g]} \times 14 \text{ [days]}}$$

4 and fetal growth rate from the first day of the protocol-defined treatment to birth (%/day) is
5 calculated using the following formula:

$$6 \quad \text{Fetal growth rate from the first day of the protocol-defined treatment to birth (\%/day)} \\ 7 = \frac{\text{Birthweight} - \text{EFW at the first day of the treatment [g]} \times 100}{\text{EFW at the first day of the treatment [g]} \times \text{Days of the treatment [days]}}$$

7 iv) Fetal head circumference (cm).

8 The fetal head circumference was measured at the plane of the third ventricle with the thalamus
9 in the central portion and the cavum septi pellucidi visible in the anterior portion.

10 v) Doppler imaging of umbilical arterial blood flow.

11 Umbilical arterial blood flow was examined by Doppler ultrasound according to the Society for
12 Maternal-Fetal Medicine (SMFM) Clinical Guidelines.[26]

13 vi) Deepest amniotic fluid pocket (cm).

14 The deepest amniotic fluid pocket was measured by transabdominal ultrasonography.

15 vii) Prolongation of GA at birth (days).

16 Prolongation of GA at birth is defined as days from the first day of the protocol-defined
17 treatment to birth.

18 viii) Birth weight (g).

19 Birth weight is defined as the weight of the infant at birth.

20 ix) GA at birth.

21 GA at birth is defined as the gestational age at birth.

22 x) Apgar score.

23 The Apgar score consists of an evaluation of five factors: heart rate, respiratory effort, muscle
24 tone, responsiveness, and color at one minute and five minutes after birth.

25 xi) Umbilical artery pH and base excess values.

26 Umbilical artery pH and base excess is measured at delivery.

27 xii) Incidence rate of pre-eclampsia.

28 Incidence rate of pre-eclampsia is defined as the percentage of enrolled patients who develop
29 pre-eclampsia after the protocol-defined treatment.

30 xiii) Pediatric developmental assessment until 1.5 years of age.

1 Pediatric developmental assessment includes physiological and neurological developmental
2 assessment, and infant complications including cerebral palsy, epilepsy, and death.

3 3) Safety endpoints

4 i) Incidence rate of obstetric complications.

5 Incidence rate of obstetric complications including hypertensive disorders of pregnancy (HDP)
6 is defined as the percentage of enrolled patients who develop obstetric complications after the
7 protocol-defined treatment.

8 ii) Perinatal mortality.

9 Perinatal mortality is defined to include stillbirths (occurring after 22 weeks of gestation) and
10 neonatal deaths (occurring up to 7 days after birth).

11 iii) Neonatal mortality.

12 Neonatal mortality is defined as neonatal deaths occurring up to 28 days after birth.

14 **Stopping Criteria**

15 The investigator must discontinue the protocol-defined treatment when certain events
16 prevent continuation of the protocol treatment. These events include the following:

- 17 1. The mother has withdrawn her consent to study participation.
- 18 2. Certain events prevent continuation of the protocol treatment, which include the following:
 - 19 a) A serious adverse drug reaction to tadalafil has developed.
 - 20 b) The investigator's decision to prioritize other management including termination of the
21 pregnancy instead of continuation of the protocol-defined treatment.
 - 22 c) The investigator's decision that it is inappropriate to continue with the protocol treatment.
 - 23 d) The mother's poor compliance or discontinuation of the protocol treatment.

25 **Criteria for Delivery**

26 In this study, to minimize bias in terms of the timing of delivery, a fetal indication for
27 delivery is established on the basis of the results from the multicenter survey of VLBW infants
28 in Japan using a network database (Figure 1 and Table 1). After registration, all patients will
29 receive the conventional management of FGR according to the guidelines for obstetrical
30 practice in Japan regardless of the treatment arm.[21] Briefly, the conventional management of
31 FGR consists of the evaluation of fetal well-being on ultrasonography, including Doppler
32 imaging of umbilical arterial blood flow, non-stress test, contraction stress test, and biophysical
33 profile scoring depending on GA, to evaluate possible pregnancy termination. The investigator
34 will evaluate the fetal condition and decide timing of delivery referring to Table 1. For other
35 complications such as preterm labor, rupture of the membranes, and hypertensive disorder of
36 pregnancy, the investigator will follow guidelines for obstetric practice in Japan.[21] The

1 investigator must provide a report that explains the reason for termination of the pregnancy on
2 the website of this clinical trial (the Clinical Trial Data Management System:
3 <http://scope.mie-cts.net/rd/p01.php>).

4 5 **Monitoring Safety during the Fetal Therapy**

6 The investigator must pay close attention to the safety of not only the fetus but also the
7 mother. As shown in the study schedule, the protocol-defined assessments include evaluation of
8 maternal blood pressure and pulse rate, maternal blood and urine tests (blood
9 fibrinogen and anti-thrombin III levels, liver and renal function tests, serum electrolyte levels,
10 and qualitative urine protein excretion), maternal serum placental growth factor (PIGF) and
11 soluble fms-like tyrosine kinase receptor (sFLT-1) levels. Other assessments include adverse
12 events assessed by medical consultation, and antepartum fetal tests consisting of
13 ultrasonography including Doppler imaging of blood flow (umbilical artery, middle cerebral
14 artery, and uterine artery), non-stress test, contraction stress test, and biophysical profile scoring
15 depending on GA. The investigator will enter patients' safety data into the Case Report Form on
16 the website of this clinical trial (the Clinical Trial Data Management System:
17 <http://scope.mie-cts.net/rd/p01.php>).

18 19 **Safety Evaluation Committee**

20 The Safety Evaluation Committee is responsible for the overall safety of this clinical
21 study. To ensure the safety of the protocol-defined treatment, the Safety Evaluation Committee
22 will review the adverse events of tadalafil treatment. If a serious adverse event develops, the
23 investigator will provide the Secretariat with the necessary information within 24 hours of its
24 onset, according to the predetermined procedure. The Secretariat then will forward the obtained
25 information without delay to the Safety Evaluation Committee for review. The Safety
26 Evaluation Committee will notify the investigator of the review results. If the adverse event is
27 definitely or probably related to tadalafil treatment, the Ethics Committee in Mie University
28 Hospital or each institute will consider possible termination of this clinical study. Special
29 attention must be paid to the reporting requirements stipulated in the Ethical Guidelines for
30 Clinical Studies (Ministry of Health, Labor, and Welfare in Japan, 2017). Infants will be
31 followed up and evaluated for physiological and neurological development until 1.5 years of
32 age.

33 34 **Sample size**

35 140 fetuses and their mothers.

36 **Rationale for the Target Sample Size**

Table 2 shows the summary of the distribution of fetal growth velocity from enrollment to birth in our retrospective study.[20] We estimate that the distribution of fetal growth velocity of this prospective phase II trial will be similar to that of our retrospective study. When the results of our prospective study are analyzed by Wilcoxon Rank Sum Test and group comparisons, with an α of 0.05, two sided, we will have 90% power to detect a difference if we randomize 62 women per group. Allowing for a 10% drop-out rate, the total sample size required is 140 women.

Table 2. The distribution of fetal growth velocity from enrollment to birth in the retrospective study conducted at Mie University Hospital.

Fetal growth velocity (g/day)	<5	≥5 to <10	≥10 to <15	≥15 to <20	≥20 to <25	≥25
The conventional management group (%)	5.3	10.5	21.1	47.3	15.8	0
The tadalafil group (%)	0	8.3	8.3	50.0	16.7	16.7

Statistical analysis

Analysis is done on all randomized fetuses who receive the protocol-defined treatment at least once, as the full analysis set. Analysis per protocol set (i.e., removing patients who do not meet the inclusion and exclusion criteria) is done as a secondary analysis population for sensitivity analysis. All outcome measures are presented as summaries of descriptive statistics (mean [SD] or median [minimum and maximum] for continuous measures, and the numbers and proportions for ordinal and dichotomous measures). Descriptive statistics for the primary endpoint, i.e. fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day), are analyzed for each treatment arm by the Wilcoxon Rank Sum Test and group comparisons. All analyses are performed according to a pre-specified statistical analysis plan. The Data Coordinating Center in Mie University Hospital supports the data management, statistical analysis, and reporting of the study.

Ethics and dissemination

This study was approved by the Institutional Review Board of Mie University Hospital on August 25th, 2016 (No.3041) prior to patient enrollment. The study protocol was also approved by each institutional review board of all participating institutions. This study complies with the Helsinki Declaration. Written informed consent will be obtained from all mothers of fetuses before they are recruited. This trial has been registered in the UMIN Clinical Trials Registry as UMIN000023778 (https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno=R000027132). Our findings will be widely disseminated through conference presentations and peer-reviewed publications.

1 **Participating institutions**

2 Mie University, Showa University, Osaka University, Nagoya University, Mie Chuo Medical
3 Center, Municipal Yokkaichi hospital, Ise Red Cross Hospital, St. Marianna University,
4 Juntendo University, the Jikei University, Toho University, Yokohama City University Medical
5 Center, Kanagawa Children's Medical Center, Ehime University, Hamamatsu University School
6 of Medicine, Osaka Medical College, Niigata University, Showa University Northern Yokohama
7 Hospital, Showa University Koto Toyosu Hospital, Gifu University, University of the Ryukyu,
8 Shiga University, Shinshu University, Ehime Prefectural Central Hospital, Akita University,
9 Tokyo Metropolitan Bokutoh Hospital, Kyorin University, Tokyo Metropolitan Tama Medical
10 Center, Kuwana East Medical Center, Kanazawa University, Nagasaki Medical Center,
11 University of Toyama, Yamaguchi University, Toyota Memorial Hospital, Kainan Hospital,
12 Dokkyo Medical University, Saga Hospital, Kyoto Prefectural University, Toyama Central
13 Prefectural Hospital, Sapporo City General Hospital, Kagoshima University, Mie Prefectural
14 General Medical Center, Kyoto University, Sakakibara Heart Institute, and University of Fukui.

15
16 The original protocol is available in the supplemental materials.

18 **DISCUSSION**

19 This protocol has been already approved by the Institutional Review Board of Mie
20 University Hospital and 39 institutions in Japan. Fetuses with FGR will be enrolled from these
21 institutions. Because fetal growth velocity from the first day of the treatment to birth has been
22 defined as the primary endpoint and fetuses will be randomly assigned in an open-label design,
23 timing of delivery should be made on the basis of similar criteria as much as possible. Each
24 participating medical center can provide treatment for fetuses with FGR by board certified
25 members of the Japan Society of Obstetrics and Gynecology, and the investigator will be able to
26 optimally decide timing of delivery according to the guidelines for obstetrical practice in
27 Japan.[21] To make more accurate decisions, a fetal indication for delivery is established in this
28 study on the basis of the results from the multicenter survey in Japan, in which 82 level III
29 perinatal centers, including 8 sites participating in this study, were registered (Table 1).[1] The
30 fetal indication for delivery is divided into three groups depending on infant survival rate in the
31 NICU. Because all patients will undergo antepartum fetal tests consisting of evaluation of fetal
32 well-being by ultrasonography, including Doppler imaging of umbilical arterial blood flow,
33 non-stress test, contraction stress test, and biophysical profile scoring depending on GA
34 according to the Japanese guidelines, the investigator will easily refer to this indication when
35 deciding timing of delivery. This indication will be used to evaluate fetal baseline condition at
36 enrollment as well. We believe that this approach could take advantage of strengths and

1 minimize the possible limitations related to open-label trial features.

2 We retrospectively compared the effect of tadalafil in patients with FGR and
3 demonstrated that both fetal growth velocity from enrollment to birth and birth weight were
4 significantly higher in the tadalafil group than in the conventional management group. The
5 required sample size of this prospective study was estimated based on the results of the
6 retrospective study that used the same primary outcome measure. Since patients with FGR were
7 enrolled in the retrospective study under similar criteria to those in this study, we think that it is
8 reasonable to use the results of the retrospective study for the estimation of sample size.

9
10 **Contributors:** T.U., S.M., M.K., H.T., M.N., K.T., K.O., Y.K., M.E., T. Kimura, T. Kotani, M.N.,
11 A.S., and T.I.: conception of the study. T.U.: writing of the manuscript. S.T., Y.N., M.K., C.M.,
12 and M.N.: providing the biostatistical study design. T.O.: statistical analysis. T. I.: principal
13 Investigator of this trial and the grant holder. All authors have read and approved the final
14 manuscript.

15
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18 Science Foundation.

19
20 **Competing interests:** None declared.

21
22 **Ethics approval:** The Institutional Review Board of of Mie University Hospital in Augst 25th,
23 2016 (No.3041).

24
25 **Data sharing statement:** There is no requirement for data sharing in public research
26 expenditures of our funds, and we are not prepared for data sharing at present. In the future, if
27 the chief researcher receives requests, we will prepare for data sharing to the extent permitted by
28 the Japanese ethics guidelines.

29
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31 Japan Society of Obstetrics and Gynecology) and Dr. Yoshiaki Miyake (Board Certified
32 Member of the Japan Society of Obstetrics and Gynecology) for their contribution as members
33 of the Safety Evaluation Committee in this trial.

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1 **REFERENCES**

- 2 1 Kusuda S, Fujimura M, Sakuma I, *et al.* Morbidity and mortality of infants with very low
3 birth weight in Japan: center variation. *Pediatrics* 2006;**118**:e1130–e1138.
- 4 2 American College of Obstetricians and Gynecologists. ACOG Practice bulletin no. 134:
5 fetal growth restriction. *Obstet Gynecol* 2013;**121**:1122–33.
- 6 3 Hui L, Challis D. Diagnosis and management of fetal growth restriction: the role of fetal
7 therapy. *Best Pract Res Clin Obstet Gynaecol* 2008;**22**:139–58.
- 8 4 Coppage KH, Sun X, Baker RS, *et al.* Expression of phosphodiesterase 5 in maternal and
9 fetal sheep. *Am J Obstet Gynecol* 2005;**193**:1005–10.
- 10 5 Rotella DP. Phosphodiesterase 5 inhibitors: current status and potential applications. *Nat*
11 *Rev Drug Discov* 2002;**1**:674–82.
- 12 6 Wareing M, Myers JE, O’Hara M, *et al.* Effects of a phosphodiesterase-5 (PDE5) inhibitor
13 on endothelium-dependent relaxation of myometrial small arteries. *Am J Obstet Gynecol*
14 2004;**190**:1283–90.
- 15 7 Wareing M, Myers JE, O’Hara M, *et al.* Sildenafil citrate (Viagra) enhances vasodilatation
16 in fetal growth restriction. *J Clin Endocrinol Metab* 2005;**90**:2550–5.
- 17 8 Herraiz S, Pellicer B, Serra V, *et al.* Sildenafil citrate improves perinatal outcome in fetuses
18 from pre-eclamptic rats. *BJOG Int J Obstet Gynaecol* 2012;**119**:1394–402.
- 19 9 Ramesar SV, Mackraj I, Gathiram P, *et al.* Sildenafil citrate improves fetal outcomes in
20 pregnant, L-NAME treated, Sprague-Dawley rats. *Eur J Obstet Gynecol Reprod Biol*
21 2010;**149**:22–6.
- 22 10 Baijnath S, Soobryan N, Mackraj I, *et al.* The optimization of a chronic nitric oxide
23 synthase (NOS) inhibition model of pre-eclampsia by evaluating physiological changes.
24 *Eur J Obstet Gynecol Reprod Biol* 2014;**182**:71–5.
- 25 11 Nassar AH, Masrouha KZ, Itani H, *et al.* Effects of sildenafil in N ω -nitro-L-arginine methyl
26 ester-induced intrauterine growth restriction in a rat model. *Am J Perinatol* 2012;**29**:429–
27 34.
- 28 12 Cross JC, Hemberger M, Lu Y, *et al.* Trophoblast functions, angiogenesis and remodeling of
29 the maternal vasculature in the placenta. *Mol Cell Endocrinol* 2002;**187**:207–12.
- 30 13 Watson ED, Cross JC. Development of structures and transport functions in the mouse
31 placenta. *Physiol Bethesda Md* 2005;**20**:180–93.
- 32 14 von Dadelszen P, Dwinnell S, Magee LA, *et al.* Sildenafil citrate therapy for severe
33 early-onset intrauterine growth restriction. *BJOG Int J Obstet Gynaecol* 2011;**118**:624–8.
- 34 15 Sharp A, Comforth C, Jackson R, *et al.* OC01.05: STRIDER UK: a randomised controlled
35 trial of sildenafil therapy in dismal prognosis early-onset intrauterine growth restriction.
36 [abstract] *Ultrasound Obstet Gynecol* 2017;**50**:3.
- 37 16 Sahni S, Palkar AV, Rochelson BL, *et al.* Pregnancy and pulmonary arterial hypertension: A
38 clinical conundrum. *Pregnancy Hypertens Int J Womens Cardiovasc Health* 2015;**5**:157–64.

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2
3
4
5 1 17 Wilkins MR, Wharton J, Grimminger F, *et al.* Phosphodiesterase inhibitors for the treatment
6 2 of pulmonary hypertension. *Eur Respir J* 2008;**32**:198–209.
- 7
8 3 18 Forgue ST, Patterson BE, Bedding AW, *et al.* Tadalafil pharmacokinetics in healthy subjects.
9 4 *Br J Clin Pharmacol* 2006;**61**:280–288.
- 10
11 5 19 Yoshikawa K, Umekawa T, Maki S, *et al.* Tadalafil Improves L-NG-Nitroarginine Methyl
12 6 Ester-Induced Preeclampsia With Fetal Growth Restriction-Like Symptoms in Pregnant
13 7 Mice. *Am J Hypertens* In press.
- 14
15 8 20 Kubo M, Umekawa T, Maekawa Y, *et al.* Retrospective study of tadalafil for fetal growth
16 9 restriction: Impact on maternal and perinatal outcomes. *J Obstet Gynaecol Res*
17 10 2017;**43**:291–297.
- 18
19 11 21 Minakami H, Maeda T, Fujii T, *et al.* Guidelines for obstetrical practice in Japan: Japan
20 12 Society of Obstetrics and Gynecology (JSOG) and Japan Association of Obstetricians and
21 13 Gynecologists (JAOG) 2014 edition. *J Obstet Gynaecol Res* 2014;**40**:1469–1499.
- 22
23 14 22 Kubo M, Tanaka H, Maki S, *et al.* Safety and dose-finding trial of tadalafil administered for
24 15 fetal growth restriction: A phase-1 clinical study. *J Obstet Gynaecol Res* 2017;**43**:1159–
25 16 1168.
- 26
27 17 23 Cunningham F, Leveno K, Bloom S, *et al.* *Williams Obstetrics, 24e.* McGraw-Hill 2014.
- 28
29 18 24 Parer JT, Ikeda T. A framework for standardized management of intrapartum fetal heart rate
30 19 patterns. *Am J Obstet Gynecol* 2007;**197**:26. e1-6.
- 31
32 20 25 Shinozuka N. Fetal biometry and fetal weight estimation: JSUM standardization.
33 21 *Ultrasound Rev Obstet Gynecol* 2002;**2**:156–161.
- 34
35 22 26 Berkley E, Chauhan SP, Abuhamad A, *et al.* Doppler assessment of the fetus with
36 23 intrauterine growth restriction. *Am J Obstet Gynecol* 2012;**206**:300–308.

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5 **FIGURE LEGENDS**

6 **Figure 1. Infant survival rate in the NICU categorized by birth weight and gestational**
7 **week at birth (%).**

8 This figure is established on the basis of the results from the multicenter survey of VLBW
9 infants in Japan using a network database. The survey data included infant survival rates in the
10 NICU, categorized by birth weight and gestational week at birth.[1] The infant survival rate data
11 acquired from the survey were preprocessed with the moving average method and divided into
12 three groups. The first group was defined as “Zone 1” where the infant survival rate in the
13 NICU was less than 60% (highlighted by a red background). The second group was defined as
14 “Zone 2” where the infant survival rate in the NICU ranged from 60 to 95% (highlighted by a
15 yellow background). The third group was defined as “Zone 3” where the infant survival rate in
16 the NICU was 95% or higher (highlighted by a blue background).
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24 **Figure 2. Summary of the study design.**
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Birth weight (g)	1401-1500						96	99	100	99	99	99	99
	1301-1400						94	97	99	99	99	100	99
	1201-1300						98	99	99	99	99	99	100
	1101-1200					96	96	99	100	99	99	99	100
	1001-1100				96	98	98	98	99	99	98	98	98
	901-1000				95	96	97	97	98	99	99	98	97
	801-900			89	91	95	96	96	97	97	98	100	100
	701-800		84	86	90	93	93	95	99	98	94	95	100
	601-700		78	86	90	93	94	93	96	100	100		
	501-600	59	69	80	90	87	93	94	92	87			
	401-500	49	64	71	80	77	80	86	100	71			
	301-400	41	52	51	56	68	67	73	71				
	201-300	18	10	31	33	40							
		22	23	24	25	26	27	28	29	30	31	32	33
		Gestational week at birth											

Figure 1

Figure 1. Infant survival rate in the NICU categorized by birth weight and gestational week at birth (%).

173x177mm (300 x 300 DPI)

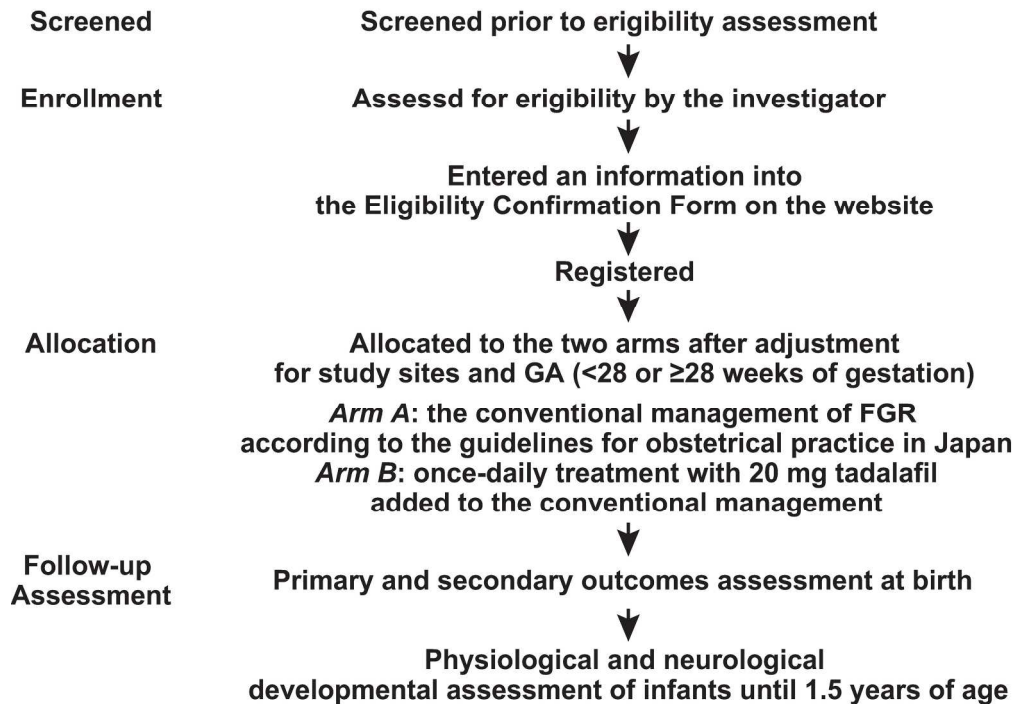


Figure 2

Figure 2. Summary of the study design.

212x193mm (300 x 300 DPI)

Study Protocol

TADAFER II:

A multicenter phase II trial of the efficacy and safety of tadalafil in fetus with early-onset growth restriction.

Trial registration: UMIN Clinical Trials Registry UMIN000023778.

Version 1

Date 25-August-2016

Contents

1		
2		
3		
4		
5		
6		
7	SYNOPSIS	3
8	1. VOLUNTARY PARTICIPATION AND WITHDRAWAL	5
9		
10	2. BACKGROUD AND OBJECTIVES	5
11		
12	3. RESEARCH ORGANIZATION AND PARTICIPATING INSTITUTIONS	8
13		
14	4. STUDY SUBJECTS AND METHODS.....	10
15		
16	5. TREATMENT AND COMPENSATION FOR STUDY-RELATED INJURY	19
17		
18	6. STUDY PERIOD AND TARGET SAMPLE SIZE	20
19		
20	7. OUTLINE OF THE STUDY PLAN.....	20
21		
22	8. ANTICIPATED ADVERSE EVENTS.....	25
23		
24	9. POTENTIAL BENEFITS AND RISKS	26
25		
26	10. BURDEN OF COST.....	27
27		
28	11. INTELLECTUAL PROPERTY RIGHTS.....	27
29		
30	12. ETHICS	27
31		
32	13. ADDITIONAL NOTES RELATED TO THE ACT ON THE PROTECTION OF PERSONAL INFORMATION RELATED BY INDEPENDENT ADMINISTRATIVE INSTITUTIONS.....	27
33		
34	14. REFERENCE	29
35		
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SYNOPSIS

1. Objectives

This multicenter randomized controlled phase II trial has been designed to prospectively evaluate the efficacy and safety of tadalafil treatment in fetus with fetal growth restriction (FGR).

2. Study eligibility

This study will include fetuses and their mothers who meet the following conditions:

- (1) Pregnant women ≥ 20 years.
- (2) Estimated fetal weight (EFW) less than 1.5 standard deviations of the mean EFW for gestational age.
- (3) Gestational age between 20 + 0 and 33 + 6 weeks.
- (4) The expected date of confinement is determined using the criteria of the guidelines for obstetrical practice in Japan (2014).¹
- (5) Singleton pregnancy.
- (6) Signed written informed consent.

3. Treatment

Fetuses with FGR will be randomized to receive either the conventional management of FGR according to the guidelines for obstetrical practice in Japan¹ or once-daily treatment with 20 mg tadalafil added to the conventional management until delivery.

4. Target sample size and duration of the study

Duration of the study: date of ethics approval to February 2021.

Target sample size: 140 singleton fetuses and their mothers.

5. Endpoints

- (1) Primary endpoint: fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day).
- (2) Secondary endpoints
 - 1) Completion rate of the treatment regimen
 - 2) Efficacy endpoints: estimated fetal weight (g), fetal growth velocity in the two weeks after the protocol-defined treatment (g/day), fetal growth velocity in the two weeks after one week of

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5 the protocol-defined treatment (g/day), fetal growth rate (%/day), fetal head circumference (cm),
6 Doppler imaging of umbilical arterial blood flow, deepest amniotic fluid pocket (cm),
7 prolongation of gestational age at birth (days), birth weight (g), gestational age (GA) at birth,
8 Apgar score, umbilical artery pH and base excess values, incidence rate of pre-eclampsia, and
9 pediatric developmental assessment until 1.5 years of age.
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13 3) Safety endpoints: incidence rate of obstetric complications, perinatal mortality, and neonatal
14 mortality.
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17 **6. Secretariats**

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1. VOLUNTARY PARTICIPATION AND WITHDRAWAL

Participation in this study is on a voluntary basis. Refusal to participate will incur no penalty or loss of benefits to which patients are otherwise entitled to. The subject may withdraw at any time without penalty.

2. BACKGROUD AND OBJECTIVES

Neonatal intensive care has improved over the past few decades, and morbidity among infants, including those who are premature, continues to decline. Premature infants with intrauterine growth restriction, however, still have high mortality and morbidity. The multicenter survey² of very low birth weight (VLBW) infants in Japan conducted by Kusuda and Ikeda *et al.* revealed that mortality in neonatal intensive care units (NICU), of small gestational age (SGA) infants born before 30 weeks gestation, was significantly higher than that of appropriate for gestational age (AGA) infants (unpublished data). To prevent fetal growth restriction (FGR), nutritional and dietary supplementation, bed rest, and aspirin therapy have been investigated, but there is insufficient evidence for the routine indication of any of these treatments.³ There is also no proven therapy to reverse or ameliorate established FGR.⁴

Increases in uteroplacental blood flow during pregnancy via angiogenesis and vasodilation contribute to adequate fetal growth. Vasodilation in the uteroplacental unit is considered to be due to the production and local release of nitric oxide (NO), which stimulates cyclic guanosine monophosphate (cGMP) production.⁵ cGMP is inactivated mainly by phosphodiesterases (PDE), and the predominant PDE isoform present in the vascular smooth muscle is PDE5. Because inhibitors of PDE5, which is a cGMP-specific PDE, exert their pharmacological action by dilating arteries and increasing blood flow, as proven in erectile dysfunction and pulmonary hypertension, recent studies have suggested a potential therapeutic role for PDE5 inhibitors in treating FGR.⁶ Sildenafil, a selective PDE5 inhibitor, has been shown to improve endothelial function in myometrial small arteries removed from women with pre-eclampsia and FGR.^{7,8} However, although sildenafil has been reported to affect maternal hypertension, it has not been shown to affect FGR in studies in FGR model rats induced by L-NG-nitroarginine methyl ester (L-NAME) treatment. In these studies, FGR was not affected by sildenafil except in one report, by Bajjnath *et al.*^{9,10,11,12} Bajjnath *et al.* demonstrated that L-NAME-induced FGR was improved by sildenafil treatment from 4 days postcoitum (d.p.c.) to

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5 8 d.p.c. but not from 8 d.p.c. to 14 d.p.c.¹¹ Chorioallantoic attachment occurs at 8 d.p.c., and the
6 mature circulatory pattern of maternal blood through the placenta is established by 10 d.p.c. in
7 mouse placenta.^{13,14} In considering the development of fetoplacental circulation in rodents, the
8 effect of sildenafil on fetal growth associated with placental blood flow via an NO-dependent
9 pathway was not manifested. In a clinical study, it was reported that sildenafil was associated
10 with increased fetal abdominal circumference (AC) growth velocity in severe early-onset FGR,
11 but the authors did not report on fetal growth velocity and birth weight.¹⁵ Recently, the
12 STRIDER UK group has found no evidence of a beneficial effect of sildenafil on survival or
13 short-term neonatal outcomes.¹⁶

14
15 Tadalafil is also a selective PDE5 inhibitor and has a longer half-life and a more rapid
16 onset of action than sildenafil.⁶ Tadalafil has been used to treat pulmonary hypertension in
17 pregnant women and the Food and Drug Administration in the United States has rated tadalafil
18 as pregnancy category B.¹⁷ When taking sildenafil with a high-fat meal, the time to maximum
19 plasma concentration increases and the peak plasma concentration falls.¹⁸ In contrast, Forgue *et*
20 *al.* reported that food intake had a negligible effect on the bioavailability of tadalafil, and also
21 reported that there was no clinically meaningful effect of gender on tadalafil
22 pharmacokinetics.¹⁹ Our animal experiments demonstrated that tadalafil treatment dilates the
23 maternal blood sinuses in the placenta, which leads to increased placental growth factor (PIGF)
24 production, and contributes to facilitating fetal growth.²⁰ Because tadalafil treatment was started
25 after blood spaces in the placenta were narrowed by L-NAME treatment and elevated urinary
26 excretion of cGMP in these animal experiments, we can safely presume that tadalafil treatment
27 contributes to facilitating fetal growth in the context of the mechanisms associated with NO
28 signaling. In addition, we retrospectively analyzed 11 Japanese singleton pregnant women with
29 FGR who received tadalafil along with conventional management for FGR at Mie University
30 Hospital from July 2015 to February 2016 (tadalafil group).²¹ These women were matched for
31 maternal age, parity, gestational age (GA), and estimated fetal weight at enrollment with 14
32 singleton pregnant women who had received only the conventional management for FGR in
33 2014 (conventional management group). The conventional management for FGR was
34 performed according to the guidelines for obstetric practice in Japan.¹ This retrospective study
35 showed that both fetal growth velocity from enrollment to birth and birth weight were
36 significantly higher in the tadalafil group than in the conventional management group.
37 Moreover, the prevalence of respiratory distress syndrome (RDS) was significantly lower in the
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tadalafil group than in the conventional management group. After the retrospective study, we conducted a phase I clinical trial to ensure the safety of tadalafil treatment for FGR.²² There were no serious maternal adverse events for daily tadalafil doses of 10 mg, 20 mg, and 40 mg. More patients who were administered 40 mg tadalafil daily experienced mild adverse events than those administered 10 mg or 20 mg tadalafil daily. In regards to fetal adverse events, intrauterine fetal death occurred in one case. In this case, the pregnant woman was prescribed 40 mg tadalafil daily and fetal growth had been progressing at a rate of 22 g/day. At 36 weeks gestation, fetal movement suddenly ceased and a diagnosis of intrauterine fetal death was made. Thereafter, the fetus was delivered vaginally, and velamentous insertion of the umbilical cord was identified. Immediately, the safety evaluation committee investigated the incident's relationship to tadalafil. This committee analyzed the case and concluded that the intrauterine fetal death was due to velamentous insertion of the umbilical cord.²³ We concluded that tadalafil treatment was feasible in pregnant women with FGR.²²

Based on the above, we have hypothesized that tadalafil therapy will safely increase the likelihood of increased fetal growth in fetuses with FGR and have designed this multicenter randomized controlled phase II study to establish evidence for fetal therapy with tadalafil. This study, funded by the Japan Agency for Medical Research and Development (AMED), will prospectively evaluate the safety and efficacy of tadalafil in FGR with the participation of major medical centers providing treatment for fetuses with FGR according to the guidelines for obstetrical practice in Japan. Fetuses will be randomized to receive either the conventional management for FGR, according to the guidelines in Japan, or a once-daily treatment with 20 mg of tadalafil along with the conventional management, until delivery. Fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day) has been defined as the primary endpoint in this study. To minimize bias in terms of fetal baseline condition and timing of delivery, a fetal indication for delivery is established on the basis of the results from the multicenter survey of VLBW infants in Japan using a network database, in which the 82 level III perinatal centers were registered.² The investigator will evaluate fetal baseline conditions at enrollment and will decide the timing of delivery based on this fetal indication. For other complications such as preterm labor, rupture of the membranes, and hypertensive disorder of pregnancy, the investigator will follow guidelines for obstetric practice in Japan.¹ The investigator will enter the patients' data into the Case Report Form on the website of this clinical trial (the Clinical Trial Data Management System: <http://scope.mie-cts.net/rd/p01.php>). Infants will be followed up and evaluated for physiological and neurological development until

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5 1.5 years of age.
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8 **3. RESEARCH ORGANIZATION AND PARTICIPATING INSTITUTIONS**

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10	(1) Corresponding	Mie University	Tomoaki Ikeda (Principal Investigator)
11	(2) Collaborator	Showa University	Akihiko Sekizawa
12		Osaka University	Tadashi Kimura
13		Nagoya University	Tomomi Kotani
14		Mie Chuo Medical Center	Yuka Maekawa
15		Municipal Yokkaichi hospital	Kenji Nagao
16		Ise Red Cross Hospital	Tomohisa Kihira
17		St. Marianna University	Nao Suzuki
18		Juntendo University	Satoru Takeda
19		The Jikei University	Aikou Okamoto
20		Toho University	Masahiko Nakata
21		Yokohama City University Medical Center	Shigeru Aoki
22		Kanagawa Children's Medical Center	Hiroshi Ishikawa
23		Ehime University	Takashi Sugiyama
24		Hamamatsu University School of Medicine	Naohiro Kanayama
25		Osaka Medical College	Masahide Ohmichi
26		Niigata University	Takayuki Enomoto
27		Showa University Northern Yokohama Hospital	Kiyotake Ichizuka
28		Showa University Koto Toyosu Hospital	Katsufumi Otsuki
29		Gifu University	Kenichiro Morishige
30		University of the Ryukyu	Yoichi Aoki
31		Shiga University	Takashi Murakami
32		Shinshu University	Tanri Shiozawa
33		Ehime Prefectural Central Hospital	Hiroshi Ochi
34		Akita University	Yukihiko Terada
35		Tokyo Metropolitan Bokutoh Hospital	Hironobu Hyodo
36		Kyorin University	Mitsutoshi Iwashita
37		Tokyo Metropolitan Tama Medical Center	Akira Kohyama
38		Kuwana East Medical Center	Yoshihito Sasaki
39		Kanazawa University	Hiroshi Fujiwara

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Nagasaki Medical Center	Ichiro Yasuhi
University of Toyama	Shigeru Saito
Yamaguchi University	Norihiro Sugino
Toyota Memorial Hospital	Hidenori Oguchi
Kainan Hospital	Tadashi Sumi
Dokkyo Medical University	Susumu Miyashita
Saga Hospital	Makoto Nomiya
Kyoto Prefectural University	Jo Kitawaki
Toyama Central Prefectural Hospital	Hiroshi Funamoto
Sapporo City General Hospital	Kazuhiko Okuyama
Kagoshima University	Hiroaki Kobayashi
Mie Prefectural General Medical Center	Hirohiko Tanaka
Kyoto University	Masaki Mandai
Sakakibara Heart Institute	Shinji Katsuragi
University of Fukui	Yoshio Yoshida

(3) Safety Evaluation Committee

The Safety Evaluation Committee is independent from research organization, and responsible for the overall safety of this clinical study. To ensure the safety of the protocol-defined treatment, the Safety Evaluation Committee will review adverse events of tadalafil. The Safety Evaluation Committee consists of Dr. Makoto Maeda (Board Certified Member of the Japan Society of Obstetrics and Gynecology) and Dr. Yoshiaki Miyake (Board Certified Member of the Japan Society of Obstetrics and Gynecology).

(4) Protocol Evaluation Committee

The Protocol Evaluation Committee is an organization of the execution of this study. All experimental protocols are evaluated and approved by the Protocol Evaluation Committee.

(5) Data Coordinating Center at the Clinical Research Support Center in Mie University Hospital

This center supports the data management, and statistical analysis and reporting of the study. This consists of Dr. Masakatsu Nishikawa (chairperson), Ms. Yuki Nishimura (data manager), and Dr. Toru Ogura (statistics).

(6) Secretariats

Dr. Takashi Umekawa, Dr. Shintaro Maki, and Dr. Michiko Kubo.

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4. STUDY SUBJECTS AND METHODS

(1) Study Sites and Subjects

1) Study Sites

This is a multicenter randomized controlled phase II trial, in which the Clinical Research Support Center in Mie University Hospital serves as the data center. Since this trial has been designed to prospectively evaluate the efficacy and safety of tadalafil treatment in FGR, fetuses will be randomized to receive either the conventional management for FGR, according to the guidelines in Japan¹ or a once-daily treatment with 20 mg of tadalafil along with the conventional management at the data center. Case registration requires the approval of the Ethics Committee. The following institutions will participate in this clinical trial:

Mie University, Showa University, Osaka University, Nagoya University, Mie Chuo Medical Center, Municipal Yokkaichi hospital, Ise Red Cross Hospital, St. Marianna University, Juntendo University, the Jikei University, Toho University, Yokohama City University Medical Center, Kanagawa Children's Medical Center, Ehime University, Hamamatsu University School of Medicine, Osaka Medical College, Niigata University, Showa University Northern Yokohama Hospital, Showa University Koto Toyosu Hospital, Gifu University, University of the Ryukyu, Shiga University, Shinshu University, Ehime Prefectural Central Hospital, Akita University, Tokyo Metropolitan Bokutoh Hospital, Kyorin University, Tokyo Metropolitan Tama Medical Center, Kuwana East Medical Center, Kanazawa University, Nagasaki Medical Center, University of Toyama, Yamaguchi University, Toyota Memorial Hospital, Kainan Hospital, Dokkyo Medical University, Saga Hospital, Kyoto Prefectural University, Toyama Central Prefectural Hospital, Sapporo City General Hospital, Kagoshima University, Mie Prefectural General Medical Center, Kyoto University, Sakakibara Heart Institute, and University of Fukui.

2) Subjects and Diagnostic Methods

All patients have to meet all inclusion criteria without violating any of the exclusion criteria listed below. All subjects will be followed-up until the end of the study.

Inclusion Criteria

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- 5 (1) Pregnant women ≥ 20 years.
- 6
- 7 (2) EFW less than 1.5 standard deviations of the mean EFW for GA.
- 8
- 9 (3) GA between 20 + 0 and 33 + 6 weeks.
- 10
- 11 (4) The expected date of confinement is determined using the criteria of the guidelines for
- 12 obstetrical practice in Japan (2014).
- 13
- 14 (5) Singleton pregnancy.
- 15
- 16 (6) Signed written informed consent.

17 **Exclusion Criteria**

- 18 (1) A result from the antepartum fetal tests, done at enrollment, which indicates that delivery
- 19 should be attempted.
- 20
- 21 (2) A history of allergy to tadalafil.
- 22
- 23 (3) Concurrent medications that interact adversely with tadalafil.
- 24
- 25 (4) Contraindication of tadalafil treatment due to renal disease.
- 26
- 27 (5) Contraindication of tadalafil treatment due to liver disease.
- 28
- 29 (6) Contraindication of tadalafil treatment due to uncontrolled arrhythmia, hypertension (BP
- 30 $>170/100$ mmHg), and hypotension (BP $<80/40$ mmHg).
- 31
- 32 (7) Fetus with suspected chromosomal disorder and/or multiple congenital anomalies.
- 33
- 34 (8) Contraindication of tadalafil treatment due to retinitis pigmentosa, coagulation defect, active
- 35 gastric and/or intestinal ulcer, and venous obstructive disease.
- 36
- 37 (9) The investigator decides to entry inappropriate.

38 **Rationale for Eligibility Criteria**

- 39 • When diagnosed as FGR, the mean EFW for GA but not the mean birthweight for GA
- 40 should be used, and the estimated date of confinement using fetal measurements obtained
- 41 during the early stage of pregnancy should be confirmed according to the guidelines for
- 42 obstetrical practice in Japan (2014) in Inclusion Criteria Nos. 2 and 4.¹
- 43
- 44 • The lower age limit (20 weeks gestation) of Inclusion Criterion No. 3 is determined referring
- 45 to the previous study protocol about the treatment for FGR.²⁴ The upper limit of <34 weeks
- 46 gestation is based on infant survival rate in the NICU categorized by birth weight and
- 47 gestational week at birth from the Japanese neonatal research network database
- 48 (<http://nponrn.umin.jp/index.html> Japanese-only website), in which indicates that treatments are
- 49 prioritized over elective delivery (Figure 1).
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						84	86	90	93	93	95	99
						78	86	90	93	94	93	96
						59	69	80	90	87	93	94
						49	64	71	80	77	80	86
						41	52	51	56	68	67	73
						18	10	31	33	40		
						22	23	24	25	26	27	28
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Figure 1. Infant survival rate in NICU assembled by birth weight and gestational week at birth (%).

This figure is established on the basis of the results from the multicenter survey of VLBW infants in Japan using a network database. The survey data included infant survival rates in the NICU, categorized by birth weight and gestational week at birth.² The infant survival rate data acquired from the survey were preprocessed with the moving average method and divided into three groups. The first group was defined as “Zone 1” where the infant survival rate in the NICU was less than 60% (highlighted by a red background). The second group was defined as “Zone 2” where the infant survival rate in the NICU ranged from 60 to 95% (highlighted by a yellow background). The third group was defined as “Zone 3” where the infant survival rate in the NICU was 95% or higher (highlighted by a blue background).

- Only singletons will be included in this study (Inclusion Criterion No. 5) to accurately evaluate clinical improvements, because fetal growth in multifetal pregnancies is different from that of singleton pregnancies.²³
- The informed consent of the mother provides the ethical basis of this study (Inclusion Criterion No. 6).
- To minimize bias in terms of fetal baseline condition at enrollment, a fetal indication for delivery is established on the basis of the results from the multicenter survey of VLBW infants in Japan using a network database, in which the 82 level III perinatal centers were registered. The survey data included infant survival rate in the NICU, categorized by birth weight and gestational week at birth (Figure 1).² The infant survival rate data acquired from the survey were preprocessed with the moving average method and divided into three groups. The first group was defined as “Zone 1” where the infant survival rate in the NICU was less than 60%. The second group was defined as “Zone 2” where the infant survival rate in the NICU ranged

from 60 to 95%. The third group was defined as “Zone 3” where the infant survival rate in the NICU was 95% or higher. All patients in our study will undergo antepartum fetal tests consisting of the evaluation of fetal well-being by ultrasonography, including Doppler imaging of umbilical arterial blood flow, non-stress test, contraction stress test, and biophysical profile scoring depending on GA, to evaluate possible pregnancy termination by the investigator at enrollment (Table 1. Exclusion Criterion No. 1).

Table 1. A fetal indication for delivery in the TADAFER II study.^{1,23,25}

Infant survival rate in the NICU (See Figure 1)	
Zone 1	Decide timing of delivery depending on available therapeutic measures at NICU in each institute.
Zone 2	Consider delivery if at least one of three findings is made, but place give high priority on the determination by the investigators. <ol style="list-style-type: none"> 1. Reversed umbilical artery blood flow during diastole. 2. Score less than 4 on the fetal biophysical profile score. 3. Feat heart rate patterns in the orange or red category for more than 30 minutes.²⁵
Zone 3	Consider delivery if at least one of five findings is made, but place give high priority on the determination by the investigators. <ol style="list-style-type: none"> 1. Reversed or absent umbilical artery blood flow during diastole. 2. Score less than 4 on the fetal biophysical profile score. (Score less than 6 on the fetal biophysical profile score if oligohydramnios is present.) 3. Feat heart rate patterns in the orange or red category for more than 30 minutes.²⁵ 4. Positive contraction stress test. 5. Impaired fetal head circumference growth for more than 2 weeks.

- Patients who have contraindications for tadalafil treatment will be excluded (Exclusion Criteria from No.2 to No.7).
- Regarding exclusion criteria No.9, this study excludes mothers with mental or psychiatric problems, since poor judgment capabilities that are often associated with such conditions may not be compatible with Inclusion Criterion No. 6.

(2) Study Design

This study is a multicenter randomized controlled phase II trial.

(3) Methods

In this multicenter clinical study, each study site will obtain ethics approval of the protocol before its implementation.

Registration

This study protocol defines all the procedures and schedules that the investigator must abide by to complete this clinical study, including patient selection and registration, fetal treatment of FGR, and follow-up (Figure 2).

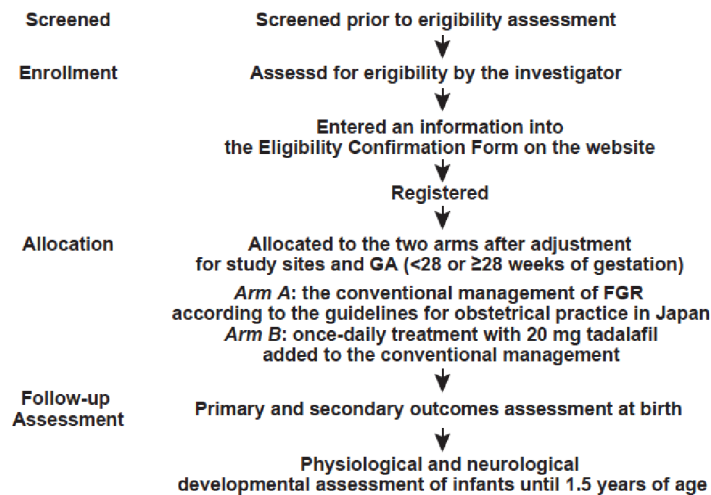


Figure 2. Summary of the study design.

The Clinical Research Support Center in Mie University Hospital will provide data center services including data management and patient registration. Patients that satisfy all inclusion criteria and do not meet any of the exclusion criteria will be eligible for inclusion in the study. Individual study sites will be responsible for guiding potential participants through the informed consent process, including patients who have been referred to them for treatment purposes. The investigator will enter an eligible patient's information into the Eligibility Confirmation Form on the website of this clinical trial (the Clinical Trial Data Management System: <http://scope.mie-cts.net/rd/p01.php> Japanese-only website). The data management system will check the contents of the form before registering the patient. For patients who meet all inclusion criteria without violating any of the exclusion criteria listed above, the data management system will register and allocate them to the two arms in an allocation ratio of 1:1, one group receiving the conventional management of FGR according to the guidelines for obstetrical practice in Japan,¹ and the other receiving once-daily treatment with 20 mg tadalafil added to conventional

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5 management after adjustment for study sites and GA (<28 or ≥28 weeks of gestation). The
6 investigators are blinded to the allocation algorithm. Enrolled participants will receive fetal
7 therapy within 7 days of registration. The investigator will enter the patients' data into the Case
8 Report Form on the website of this clinical trial (the Clinical Trial Data Management System:
9 <http://scope.mie-cts.net/rd/p01.php>).

10 The corresponding researcher at Mie University will be responsible for the management of this
11 study (patient registration, data management, and coordination with the study-related
12 committees and the Clinical Research Support Center in Mie University Hospital). The
13 corresponding researcher will also be responsible for the research administration, scheduling,
14 documentation, and safety information management. The Safety Evaluation Committee will
15 assume responsibility for the safety of this study. The Clinical Research Support Center in Mie
16 University Hospital will provide technical support from the planning to the completion of this
17 clinical study. Its Data Management Department will manage the study data in cooperation with
18 the corresponding researcher and secretariats, and its Statistics Department will provide
19 statistical support to facilitate the efficacy evaluation. The Protocol Evaluation Committee is an
20 organization of the execution of this study. All experimental protocols are evaluated and
21 approved by the Protocol Evaluation Committee.
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33 **UMIN Clinical Trials Registry UMIN000023778.**

34 **Fetal Treatment Protocol**

35 The investigator will provide the fetal therapy as described below.

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37 *Arm A:* the conventional management of FGR according to the guidelines for obstetrical
38 practice in Japan.¹ Briefly, the conventional management of FGR consists of evaluation of fetal
39 well-being on ultrasonography, including Doppler imaging of umbilical arterial blood flow,
40 non-stress test, contraction stress test, and biophysical profile scoring depending on GA to
41 evaluate possible pregnancy termination.
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47 *Arm B:* once-daily treatment with 20 mg tadalafil added to the conventional management until
48 delivery.
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50 The investigators are blinded to the allocation algorithm. Enrolled participants will receive fetal
51 therapy within 7 days of registration.
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54 **Rationale for Dose Selection**

55 Tadalafil was approved for treatment of erectile dysfunction (ED) in July 2007 in Japan.
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5 Nishiuma S *et al.* reported the results from a post marketing surveillance study on tadalafil, with
6 a primary goal of confirming the safety and effectiveness of tadalafil in Japanese patients with
7 ED in routine clinical practice. 86.7 % of the participants in the surveillance study were
8 prescribed 10mg or 20mg tadalafil daily.²⁶ We referred the results of adverse events in the
9 surveillance study and determined the dose of tadalafil in our retrospective study, in which three
10 pregnant women (27.3%) were prescribed 10 mg tadalafil daily and eight pregnant women
11 (72.7%) were prescribed 20 mg daily.²¹ In our phase I study, more patients who were
12 administered 40 mg tadalafil daily experienced adverse events than those administered 10 mg or
13 20 mg tadalafil daily, but we found that there were no serious maternal adverse events.²² Finally,
14 the minimum required sample size was estimated based on the results of our retrospective study.
15 Taken together, the tadalafil dosage (once-daily treatment with 20 mg) was set in this study.
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24 **Stopping Criteria**

25 The investigator must discontinue the protocol-defined treatment when certain events prevent
26 continuation of the protocol treatment. These events include the following:
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- 28 1. The mother has withdrawn her consent to study participation.
- 29 2. Certain events prevent continuation of the protocol treatment, which include the following:
 - 30 a) A serious adverse drug reaction to tadalafil has developed.
 - 31 b) The investigator's decision to prioritize other management including termination of the
 - 32 pregnancy instead of continuation of the protocol-defined treatment.
 - 33 c) The investigator's decision that it is inappropriate to continue with the protocol treatment.
 - 34 d) The mother's poor compliance or discontinuation of the protocol treatment.

35 Fetuses in whom the fetal therapy was prematurely discontinued as per the Stopping Criteria
36 will receive scheduled examinations and other assessments to the extent possible. If the mother
37 withdraws her consent to study participation, she and her fetus will be removed from the study.
38 If the fetal therapy is prematurely discontinued due to a serious adverse drug reaction to
39 tadalafil, scheduled subsequent examinations and other assessments should be continued to the
40 extent possible and the investigator should provide the patient experiencing an adverse event
41 with the most appropriate therapeutic measures available. If a registered mother or her fetus is
42 found to have been non-conformant to the eligibility criteria, poor compliance and dropping out
43 with the protocol treatment, the mother or fetus will be categorized as noncompliant.
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54 **Criteria for Delivery**

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5 In this study, to minimize bias in terms of the timing of delivery, a fetal indication for delivery
6 is established on the basis of the results from the multicenter survey of VLBW infants in Japan
7 using a network database (Figure 1 and Table 1). After registration, all patients will receive the
8 conventional management of FGR according to the guidelines for obstetrical practice in Japan
9 regardless of the treatment arm.¹ Briefly, the conventional management of FGR consists of the
10 evaluation of fetal well-being on ultrasonography, including Doppler imaging of umbilical
11 arterial blood flow, non-stress test, contraction stress test, and biophysical profile scoring
12 depending on GA, to evaluate possible pregnancy termination. The investigator will evaluate
13 the fetal condition and decide timing of delivery referring to Table 1. For other complications
14 such as preterm labor, rupture of the membranes, and hypertensive disorder of pregnancy, the
15 investigator will follow guidelines for obstetric practice in Japan.¹ The investigator must
16 provide a report that explains the reason for termination of the pregnancy on the website of this
17 clinical trial (the Clinical Trial Data Management System: <http://scope.mie-cts.net/rd/p01.php>).
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27 **Monitoring Safety during the Fetal Therapy**

28 The investigator must pay close attention to the safety of not only the fetus but also the mother.
29 As shown in the study schedule, the protocol-defined assessments include evaluation of
30 maternal blood pressure and pulse rate, maternal blood and urine tests (blood
31 fibrinogen and anti-thrombin III levels, liver and renal function tests, serum electrolyte levels,
32 and qualitative urine protein excretion), maternal serum placental growth factor (PlGF) and
33 soluble fms-like tyrosine kinase receptor (sFLT-1) levels. Other assessments include adverse
34 events assessed by medical consultation, and antepartum fetal tests consisting of
35 ultrasonography including Doppler imaging of blood flow (umbilical artery, middle cerebral
36 artery, and uterine artery), non-stress test, contraction stress test, and biophysical profile scoring
37 depending on GA. The investigator will enter patients' safety data into the Case Report Form on
38 the website of this clinical trial (the Clinical Trial Data Management System:
39 <http://scope.mie-cts.net/rd/p01.php>).
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49 **Safety Evaluation Committee**

50 The Safety Evaluation Committee is responsible for the overall safety of this clinical study. To
51 ensure the safety of the protocol-defined treatment, the Safety Evaluation Committee will
52 review the adverse events of tadalafil treatment. If a serious adverse event develops, the
53 investigator will provide the Secretariat with the necessary information within 24 hours of its
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5 onset, according to the predetermined procedure. The Secretariat then will forward the obtained
6 information without delay to the Safety Evaluation Committee for review. The Safety
7 Evaluation Committee will notify the investigator of the review results. If the adverse event is
8 definitely or probably related to tadalafil treatment, the Ethics Committee in Mie University
9 Hospital or each institute will consider possible termination of this clinical study. Special
10 attention must be paid to the reporting requirements stipulated in the Ethical Guidelines for
11 Clinical Studies (Ministry of Health, Labor, and Welfare in Japan, 2017). Infants will be
12 followed up and evaluated for physiological and neurological development until 1.5 years of
13 age.
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21 **Note for New Participating Study Sites**

22 This multicenter study is open to new study sites. It is desirable that study sites cooperate with
23 each other. Agreement on this inter-institutional cooperation is a prerequisite for participation in
24 this clinical study. Case registration requires the approval of the Ethics Committee in each
25 institute.
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5. TREATMENT AND COMPENSATION FOR STUDY-RELATED INJURY

Based on our previous studies, we do not expect that serious adverse events will occur frequently in this study.²² However, the investigator may encounter such adverse events as those mentioned in Section 8: Anticipated Adverse Events. The investigator must report adverse drug reactions to the Minister of Health, Labour and Welfare as provided in the Pharmaceuticals and Medical Devices Act. The investigator must also report any serious adverse events without delay to the head of his or her institution, who will in turn forward the information to the Secretariat. The Secretariat will inform the participating study sites of all reported serious adverse events, irrespective of whether expected or unexpected. The Safety Evaluation Committee will review serious adverse event reports and make recommendations to the Principal Investigator, as appropriate. More specifically, the Safety Evaluation Committee will review the information on a serious adverse event that the investigator forwarded as per the predetermined procedure to the Secretariat within 24 hours of its onset. The Safety Evaluation Committee will notify the review results to the investigator. If the adverse event is definitely or probably related to tadalafil treatment, the Ethics Committee in Mie University Hospital or each institute will consider possible termination of this clinical study. Special attention must be paid to the reporting requirements stipulated in the Ethical Guidelines for Clinical Studies (Ministry of Health, Labor, and Welfare in Japan, 2017).

According to the provisions of the Ethical Guidelines for Clinical Studies (Ministry of Health, Labour and Welfare in Japan, 2017), the study site will inform the Ministry of Health, Labour and Welfare of unexpected adverse events whose study causality cannot be denied. The Ministry of Health, Labour and Welfare will announce reported serious adverse drug reactions to the public at regular intervals. The study site must provide the patient experiencing an adverse event with the most appropriate therapeutic measures available. In this clinical study, maternal complications associated with the protocol-defined treatment have been covered by liability insurance. However, because fetal complications associated with the protocol-defined treatment have not been covered by liability insurance, the investigator must describe this issue in the informed consent document. The corresponding researcher at Mie University is responsible for dealing with inquiries from participating study sites. In case of an accident, the corresponding researcher will consult the Ethics Committee in Mie University for guidance. This study will comply with the reporting requirements provided in the Ethical Guidelines for Clinical Studies (Ministry of Health, Labour and Welfare in Japan, 2017).

6. STUDY PERIOD AND TARGET SAMPLE SIZE

(1) Study Period

The planned study period is from date of ethics approval to February 2021. The Patient Registration Period will last until December 2018. The children's outcome will be followed up for 1.5 years after birth. Data collected by the end of the Neonatal Evaluation Period will be subjected to statistical analysis.

Patient Registration Period: date of ethics approval to December 2018.

Children's Outcome Follow-up Period: 1.5 years after the last birth

(2) Target Sample Size

140 fetuses and their mothers

Rationale for the Target Sample Size

Table 2 shows the summary of the distribution of fetal growth velocity from enrollment to birth in our retrospective study.²¹ We estimate that the distribution of fetal growth velocity of this prospective phase II trial will be similar to that of our retrospective study. When the results of our prospective study are analyzed by Wilcoxon Rank Sum Test and group comparisons, with an α of 0.05, two sided, we will have 90% power to detect a difference if we randomize 62 women per group. Allowing for a 10% drop-out rate, the total sample size required is 140 women.

Table 2. The distribution of fetal growth velocity from enrollment to birth in the retrospective study conducted at Mie University Hospital.

Fetal growth velocity (g/day)	<5	≥5 to <10	≥10 to <15	≥15 to <20	≥20 to <25	≥25
The conventional management group (%)	5.3	10.5	21.1	47.3	15.8	0
The tadalafil group (%)	0	8.3	8.3	50.0	16.7	16.7

7. OUTLINE OF THE STUDY PLAN

1. The investigator will register patients with the Clinical Trial Data Management System (<http://scope.mie-cts.net/rd/p01.php>) according to the procedure defined above.
2. The Clinical Trial Data Management System will register and allocate them to the two arms in an allocation ratio of 1:1, one group receiving the conventional management of FGR according to the guidelines for obstetrical practice in Japan,¹ and the other receiving once-daily treatment with 20 mg tadalafil added to conventional management after adjustment for study sites and GA (<28 or ≥28 weeks of gestation).

3. The investigator will conduct the protocol-defined treatment. The Stopping Criteria and the Criteria for Delivery are explained in detail above.

4. Timing and Methods of Evaluation

The investigator will evaluate the variables listed below according to the study schedule. The investigator will use the Case Report Form on the website of this clinical trial (the Clinical Trial Data Management System: <http://scope.mie-cts.net/rd/p01.php>).

5. Variables

The following safety and efficacy variables will be statistically analyzed:

Variables

(1) Maternal and Fetal

i) Signs and symptoms

Headache, vertigo, flushing, epistaxis, palpitations, anorexia, dyspepsia, diarrhea, nausea, myalgia, arthralgia, dyspnea, and fetal movement counting.

ii) Maternal vital signs

Blood pressure and pulse rate.

iii) Maternal blood and urine test

Complete blood count, blood fibrinogen and anti-thrombin 3 levels, liver and renal function tests, serum electrolyte levels, qualitative urine protein excretion, maternal serum placental growth factor (PIGF), and soluble fms-like tyrosine kinase receptor (sFLT-1) levels.

iv) Fetal ultrasound examination

Estimated fetal weight (g), fetal head circumference (cm), deepest amniotic fluid pocket (cm), Doppler imaging of blood flow (umbilical artery, middle cerebral artery, and uterine artery)

v) Obstetrics

Onset of obstetrical complications including hypertensive disorder of pregnancy (HDP), treatment for obstetrical complications, indication for delivery, mode of delivery, and placental weight.

vi) Compliance of tadalafil treatment (arm B only).

vi) Adverse events

(2) Neonatal

i) GA at birth.

ii) Physical development

Body weight, height, head circumference, and percentile of birth weight for GA and sex

iii) Apgar score

iv) Clinical laboratory testing

Umbilical artery pH and base excess values

v) Admission in the NICU

vi) Neonatal complications

Respiratory distress syndrome (RDS), pulmonary hemorrhage, neonatal pulmonary hypertension, neonatal chronic lung disease, symptomatic patent ductus arteriosus (PDA), late-onset circulatory dysfunction, intraventricular hemorrhage, periventricular leukomalacia, hypoxic-ischemic encephalopathy, sepsis, necrotizing enterocolitis, gastroesophageal reflux, meconium plug syndrome, retinopathy of prematurity (ROP), anemia of prematurity, auditory disorder (abnormal auditory brainstem response results), congenital abnormality, death, and others.

(3) Pediatric

Physiological and neurological developmental assessment until 1.5 years of age, infant complications including cerebral palsy and epilepsy, and death.

Study Endpoints

(1) Primary endpoint

Fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day).

The primary endpoint is fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day), and is calculated using the following formula:

$$\text{Fetal growth velocity (g/day)} = \frac{\text{Birthweight} - \text{EFW at the first day of the treatment [g]}}{\text{Days of the treatment [days]}}$$

Rationale for the primary endpoint

Our primary hypothesis is that tadalafil therapy will increase the likelihood of increased fetal growth velocity in fetuses with FGR. Taking into account the results of our retrospective study demonstrating that tadalafil treatment increased fetal growth velocity (g/day), which was the primary outcome of the retrospective study, and decreased the incidence of RDS, an improvement in fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day) is an important indicator of the therapeutic benefits for fetuses with FGR.²¹

(2) Secondary endpoints

1) Completion rate of the treatment regimen.

Completion rate of the treatment regimen is defined as the percentage of enrolled patients who receive the protocol-defined treatment for more than 7 days.

2) Efficacy endpoints.

i) Estimated fetal weight (g).

Estimated fetal weight (EFW) is calculated using the following formula:²⁷

$$\text{EFW (g)} = 1.07 \times (\text{biparietal diameter: BPD})^3 + 0.3 \times (\text{abdominal circumference: AC})^2 \times (\text{femur length: FL})$$

ii) Fetal growth velocity in the two weeks after the protocol-defined treatment and in the two weeks after one week of the protocol-defined treatment (g/day).

Fetal growth velocity in the two weeks after the protocol-defined treatment (g/day) is calculated using the following formula:

$$\begin{aligned} & \text{Fetal growth velocity in the two weeks after the protocol – defined treatment (g/day)} \\ &= \frac{(\text{EFW two weeks after the treatment} - \text{EFW at the first day of the treatment [g]})}{14 \text{ [days]}} \end{aligned}$$

and fetal growth velocity in the two weeks after one week of the protocol-defined treatment (g/day) is calculated using the following formula:

$$\begin{aligned} & \text{Fetal growth velocity in the two weeks after one week of the treatment (g/day)} \\ &= \frac{(\text{EFW three weeks after the treatment} - \text{EFW one week after the treatment [g]})}{14 \text{ [days]}} \end{aligned}$$

iii) Fetal growth rate in the two weeks after the protocol-defined treatment and from the first day of the protocol-defined treatment to birth (%/day).

Fetal growth rate in the two weeks after the protocol-defined treatment (%/day) is calculated using the following formula:

$$\begin{aligned} & \text{Fetal growth rate in the two weeks after the protocol-defined treatment (%/day)} \\ &= \frac{\text{EFW two weeks after the treatment} - \text{EFW at the first day of the treatment [g]}}{\text{EFW at the first day of the treatment [g]}} \times 100 \\ &= \frac{\quad}{14 \text{ [days]}} \end{aligned}$$

and Fetal growth rate from the first day of the protocol-defined treatment to birth (%/day) is calculated using the following formula:

$$\begin{aligned} & \text{Fetal growth rate from the first day of the protocol-defined treatment to birth (%/day)} \\ &= \frac{\text{Birthweight} - \text{EFW at the first day of the treatment [g]}}{\text{EFW at the first day of the treatment [g]}} \times 100 \\ &= \frac{\quad}{\text{Days of the treatment [days]}} \end{aligned}$$

iv) Fetal head circumference (cm).

The fetal head circumference was measured at the plane of the third ventricle with the thalamus

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5 in the central portion and the cavum septi pellucidi visible in the anterior portion.

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7 v) Doppler imaging of umbilical arterial blood flow.

8 Umbilical arterial blood flow was examined by Doppler ultrasound according to the Society for
9 Maternal-Fetal Medicine (SMFM) Clinical Guideline.²⁸

10
11 vi) Deepest amniotic fluid pocket (cm).

12 The deepest amniotic fluid pocket was measured by transabdominal ultrasonography.

13
14 vii) Prolongation of gestational age at birth (days).

15 Prolongation of gestational age at birth is defined as days from the first day of the
16 protocol-defined treatment to birth.

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18 viii) Birth weight (g).

19 Birth weight is defined as the weight of the infant at birth.

20
21 ix) GA at birth.

22 GA at birth is defined as the gestational age at birth.

23
24 x) Apgar score.

25 The Apgar score consists of an evaluation of five factors: heart rate, respiratory effort, muscle
26 tone, responsiveness and color at one minute and five minutes after birth.

27
28 xi) Umbilical artery pH and base excess values.

29 Umbilical artery pH and base excess is measured at delivery.

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31 xii) Incidence rate of pre-eclampsia.

32 Incidence rate of pre-eclampsia is defined as the percentage of enrolled patients who develop
33 pre-eclampsia after the protocol-defined treatment.

34
35 xiii) Pediatric developmental assessment until 1.5 years of age.

36 Pediatric developmental assessment includes physiological and neurological developmental
37 assessment, and infant complications including cerebral palsy, epilepsy, and death.

38
39 3) Safety endpoints

40 i) Incidence rate of obstetric complications.

41 Incidence rate of obstetric complications including HDP is defined as the percentage of enrolled
42 patients who develop obstetric complications after the protocol-defined treatment.

43
44 ii) Perinatal mortality.

45 Perinatal mortality is defined to include stillbirths (occurring after 22 weeks of gestation) and
46 neonatal deaths (occurring up to 7 days after birth).

47
48 iii) Neonatal mortality.

49 Neonatal mortality is defined as neonatal deaths occurring up to 28 days after birth.

(3) Statistics

Analysis is done on all randomized fetuses who receive the protocol-defined treatment at least once, as the full analysis set. Analysis per protocol set (i.e., removing patients who do not meet the inclusion and exclusion criteria) is done as a secondary analysis population for sensitivity analysis. All outcome measures are presented as summaries of descriptive statistics (mean [SD] or median [minimum and maximum] for continuous measures, and the numbers and proportions for ordinal and dichotomous measures). Descriptive statistics for the primary endpoint, i.e. fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day), are analyzed for each treatment arm by the Wilcoxon Rank Sum Test and group comparisons. All analyses are performed according to a pre-specified statistical analysis plan. The Data Coordinating Center in Mie University Hospital supports the data management, statistical analysis, and reporting of the study.

8. ANTICIPATED ADVERSE EVENTS

Because we have already demonstrated in phase I clinical trial that tadalafil treatment was feasible in pregnant women with FGR,²² tadalafil treatment for FGR can be administered with relative safety and ease. Yet, this therapy may give rise to unexpected adverse events, given the limited clinical experience with this approach and exposure of healthy mothers without pulmonary hypertension to tadalafil. The investigator must fully inform prospective participants of such possibility and administer the fetal therapy with careful attention and monitoring. Adverse reactions to tadalafil divided into the four groups by the frequency (Very common [$\geq 1/10$], common [$\geq 1/100$ to $< 1/10$], uncommon [$\geq 1/1,000$ to $< 1/100$], and not known [cannot be estimated from the available data]) described in the product information of tadalafil (ADCIRCA® 20 mg tablets) are shown below:²⁹

- Very common ($\geq 1/10$)
Headache, flushing, nasopharyngitis, nausea, dyspepsia, myalgia, neck pain, and pain in extremity.
- Common ($\geq 1/100$ to $< 1/10$)
Hypersensitivity reactions*, syncope, migraine*, blurred vision, palpitations* ***, hypotension, epistaxis, vomiting, gastroesophageal reflux, rash, increased uterine bleeding**, facial oedema, and chest pain***.
- Uncommon ($\geq 1/1,000$ to $< 1/100$)

10. BURDEN OF COST

This research was supported by by the Japan Agency for Medical Research and Development (AMED). This fund will be paid for items related to research (purchasing cost for tadalafil, data management, storage, analysis, etc.) other than medical examination. Medical examination expenses are covered by the national health insurance scheme.

11. INTELLECTUAL PROPERTY RIGHTS

Any intellectual property rights that may arise from this clinical study shall be exclusively owned by the TADAFER study group. The corresponding researcher and the joint researchers report no conflicts of interest related to this clinical study or to their organizations.

12. ETHICS

This clinical study focuses on prenatal treatment, and its protocol has been developed according to the Ethical Guidelines for Clinical Studies (Ministry of Health, Labour and Welfare in Japan, 2017). Before the start of this clinical study, the corresponding researcher will explain its objectives and outline them fully to the participating site investigators. We believe that application of the guideline requirements to the mother who consents to participate in this study will ensure that her fetus is also protected by the ethical principles of the guidelines. As per the Ethical Guidelines for Clinical Studies, participation in this study will be preceded by the informed consent process. Considering the difficulty in obtaining assent, even implicitly, from the fetus, we believe that the parental permission for the fetus to participate.

13. ADDITIONAL NOTES RELATED TO THE ACT ON THE PROTECTION OF PERSONAL INFORMATION RELATED BY INDEPENDENT ADMINISTRATIVE INSTITUTIONS

1. Data Collection

Study data will be de-identified before being stored in electronic format. De-identified or anonymous data will be analyzed at Mie University. Joint researchers will examine and discuss the analyzed results.

2. Data Management

The results of analyses of the collected test data will be securely stored at the Secretariat located in Mie University.

3. Storage of Electronic Media

The results of analyses will be filed in electronic media, which will be kept securely in a locked room of Mie University. The Secretariat staff member, Dr. Takashi Umekawa, assumes the responsibility for data storage. In addition to the corresponding researcher, appointed members of the Secretariat staff will be granted access to the study data.

4. Method and Timing of Data De-identification

Registration numbers will be used to de-identify the study data at individual study sites. Each study site must ensure that the data they transfer to the Secretariat contains no explicit personal identifiers.

5. Notification of Analytical Results

Parents who participate in this study will not be informed of the results of this study.

14. REFERENCE

- 1 Minakami H, Maeda T, Fujii T, Hamada H, Iitsuka Y, Itakura A *et al.*
2 Guidelines for obstetrical practice in Japan: Japan Society of Obstetrics and Gynecology
3 (JSOG) and Japan Association of Obstetricians and Gynecologists (JAOG) 2014 edition. *J*
4 *Obstet Gynaecol Res* 2014; **40**: 1469–1499.
- 5
6
7
8
9
10
11
12
13 2 Kusuda S, Fujimura M, Sakuma I, Aotani H, Kabe K, Itani Y *et al.*
14 Morbidity and mortality of infants with very low birth weight in Japan: center variation.
15 *Pediatrics* 2006; **118**: e1130–e1138.
- 16
17
18 3 American College of Obstetricians and Gynecologists. ACOG Practice
19 bulletin no. 134: fetal growth restriction. *Obstet Gynecol* 2013; **121**: 1122–1133.
- 20
21 4 Hui L, Challis D. Diagnosis and management of fetal growth restriction: the
22 role of fetal therapy. *Best Pract Res Clin Obstet Gynaecol* 2008; **22**: 139–158.
- 23
24 5 Coppage KH, Sun X, Baker RS, Clark KE. Expression of phosphodiesterase
25 5 in maternal and fetal sheep. *Am J Obstet Gynecol* 2005; **193**: 1005–1010.
- 26
27 6 Rotella DP. Phosphodiesterase 5 inhibitors: current status and potential
28 applications. *Nat Rev Drug Discov* 2002; **1**: 674–682.
- 29
30 7 Wareing M, Myers JE, O’Hara M, Kenny LC, Warren AY, Taggart MJ *et al.*
31 Effects of a phosphodiesterase-5 (PDE5) inhibitor on endothelium-dependent relaxation of
32 myometrial small arteries. *Am J Obstet Gynecol* 2004; **190**: 1283–1290.
- 33
34
35 8 Wareing M, Myers JE, O’Hara M, Baker PN. Sildenafil citrate (Viagra)
36 enhances vasodilatation in fetal growth restriction. *J Clin Endocrinol Metab* 2005; **90**: 2550–
37 2555.
- 38
39
40 9 Herraiz S, Pellicer B, Serra V, Cauli O, Cortijo J, Felipe V *et al.* Sildenafil
41 citrate improves perinatal outcome in fetuses from pre-eclamptic rats. *BJOG Int J Obstet*
42 *Gynaecol* 2012; **119**: 1394–1402.
- 43
44
45 10 Ramesar SV, Mackraj I, Gathiram P, Moodley J. Sildenafil citrate improves
46 fetal outcomes in pregnant, L-NAME treated, Sprague-Dawley rats. *Eur J Obstet Gynecol*
47 *Reprod Biol* 2010; **149**: 22–26.
- 48
49
50 11 Baijnath S, Soobryan N, Mackraj I, Gathiram P, Moodley J. The
51 optimization of a chronic nitric oxide synthase (NOS) inhibition model of pre-eclampsia by
52 evaluating physiological changes. *Eur J Obstet Gynecol Reprod Biol* 2014; **182**: 71–75.
- 53
54
55 12 Nassar AH, Masrouha KZ, Itani H, Nader KA, Usta IM. Effects of sildenafil
56 in N ω -nitro-L-arginine methyl ester-induced intrauterine growth restriction in a rat model. *Am J*
57

- 1
2
3
4
5
6 *Perinatol* 2012; **29**: 429–434.
- 7 13 Cross JC, Hemberger M, Lu Y, Nozaki T, Whiteley K, Masutani M *et al*.
8 Trophoblast functions, angiogenesis and remodeling of the maternal vasculature in the placenta.
9 *Mol Cell Endocrinol* 2002; **187**: 207–212.
- 10 14 Watson ED, Cross JC. Development of structures and transport functions in
11 the mouse placenta. *Physiol Bethesda Md* 2005; **20**: 180–193.
- 12 15 von Dadelszen P, Dwinnell S, Magee LA, Carleton BC, Gruslin A, Lee B *et*
13 *al*. Sildenafil citrate therapy for severe early-onset intrauterine growth restriction. *BJOG Int J*
14 *Obstet Gynaecol* 2011; **118**: 624–628.
- 15 16 Sharp A, Comforth C, Jackson R, Turner M, Kenny L, Baker P *et al*.
16 OC01.05: STRIDER UK: a randomised controlled trial of sildenafil therapy in dismal prognosis
17 early-onset intrauterine growth restriction. *Ultrasound Obstet Gynecol* 2017; **50**: 3–3.
- 18 17 Sahni S, Palkar AV, Rochelson BL, Keça W, Talwar A. Pregnancy and
19 pulmonary arterial hypertension: A clinical conundrum. *Pregnancy Hypertens Int J Womens*
20 *Cardiovasc Health* 2015; **5**: 157–164.
- 21 18 Wilkins MR, Wharton J, Grimminger F, Ghofrani HA. Phosphodiesterase
22 inhibitors for the treatment of pulmonary hypertension. *Eur Respir J* 2008; **32**: 198–209.
- 23 19 Forgue ST, Patterson BE, Bedding AW, Payne CD, Phillips DL, Wrishko
24 RE *et al*. Tadalafil pharmacokinetics in healthy subjects. *Br J Clin Pharmacol* 2006; **61**: 280–
25 288.
- 26 20 Yoshikawa K, Umekawa T, Maki S, Kubo M, Nii M, Tanaka K *et al*.
27 Tadalafil Improves L-NG-Nitroarginine Methyl Ester-Induced Preeclampsia With Fetal Growth
28 Restriction-Like Symptoms in Pregnant Mice. *Am J Hypertens In press*.
- 29 21 Kubo M, Umekawa T, Maekawa Y, Tanaka H, Nii M, Murabayashi N *et al*.
30 Retrospective study of tadalafil for fetal growth restriction: Impact on maternal and perinatal
31 outcomes. *J Obstet Gynaecol Res* 2017; **43**: 291–297.
- 32 22 Kubo M, Tanaka H, Maki S, Nii M, Murabayashi N, Osato K *et al*. Safety
33 and dose-finding trial of tadalafil administered for fetal growth restriction: A phase-1 clinical
34 study. *J Obstet Gynaecol Res* 2017; **43**: 1159–1168.
- 35 23 Cunningham F, Leveno K, Bloom S, Spong CY, Dashe J. *Williams*
36 *Obstetrics, 24e*. McGraw-Hill, 2014.
- 37 24 Ganzevoort W, Alfirevic Z, von Dadelszen P, Kenny L, Papageorgiou A,
38 van Wassenaer-Leemhuis A *et al*. STRIDER: Sildenafil Therapy In Dismal prognosis

1
2
3
4
5 Early-onset intrauterine growth Restriction—a protocol for a systematic review with individual
6 participant data and aggregate data meta-analysis and trial sequential analysis. *Syst Rev* 2014; **3**:
7 23.

8
9
10 25 Parer JT, Ikeda T. A framework for standardized management of intrapartum
11 fetal heart rate patterns. *Am J Obstet Gynecol* 2007; **197**: 26–e1.

12
13 26 Shinichi Nishiuma, Kimiko Arakawa, Masanori Taketsuna, Nobuyuki
14 Kobayashi. Safety and effectiveness of tadalafil in patients with erectile dysfunction based on
15 post marketing surveillance study. *Jpn J Impot Res* 2012; **27**: 15–26.

16
17
18 27 Shinozuka N. Fetal biometry and fetal weight estimation: JSUM
19 standardization. *Ultrasound Rev Obstet Gynecol* 2002; **2**: 156–161.

20
21 28 Berkley E, Chauhan SP, Abuhamad A, Committee S for M-FMP. Doppler
22 assessment of the fetus with intrauterine growth restriction. *Am J Obstet Gynecol* 2012; **206**:
23 300–308.

24
25 29 ADCIRCA, INN - Tadalafil - WC500032789.pdf.
26 [http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/huma](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/001021/WC500032789.pdf)
27 [n/001021/WC500032789.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/001021/WC500032789.pdf) (accessed 19 Nov2017).
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CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4-6
	2b	Specific objectives or hypotheses	6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6-8
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	N/A
Participants	4a	Eligibility criteria for participants	6-7
	4b	Settings and locations where the data were collected	8 and 14
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6-8 and 11
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	8-11
	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample size	7a	How sample size was determined	13
	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	8
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	8
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	8
	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	8
Implementation	11	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	N/A

	11	If relevant, description of the similarity of interventions	8
	b		
Statistical methods	12	Statistical methods used to compare groups for primary and secondary outcomes	13
	12	Methods for additional analyses, such as subgroup analyses and adjusted analyses	N/A
Results			
Participant flow (a diagram is strongly recommended)	13	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	N/A
	13	For each group, losses and exclusions after randomisation, together with reasons	N/A
Recruitment	14	Dates defining the periods of recruitment and follow-up	N/A
	14	Why the trial ended or was stopped	N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	N/A
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	N/A
Outcomes and estimation	17	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	N/A
	17	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	N/A
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	N/A
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	N/A
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	14-15
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	14-15
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	14-15
Other information			
Registration	23	Registration number and name of trial registry	13
Protocol	24	Where the full trial protocol can be accessed, if available	N/A
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	15

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

BMJ Open

TADAFER II: Tadalafil treatment for fetal growth restriction - a study protocol for a multicenter randomized controlled phase II trial.

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-020948.R1
Article Type:	Protocol
Date Submitted by the Author:	06-Feb-2018
Complete List of Authors:	Umekawa, Takashi; Mie University Graduate School of Medicine, Obstetrics and Gynecology Maki, Shintaro; Mie University Graduate School of Medicine, Obstetrics and Gynecology Kubo, Michiko; Mie University Graduate School of Medicine, Obstetrics and Gynecology Tanaka, Hiroaki; Mie University Graduate School of Medicine, Obstetrics and Gynecology Nii, Masafumi; Mie University Graduate School of Medicine, Obstetrics and Gynecology Tanaka, Kayo; Mie University Graduate School of Medicine, Obstetrics and Gynecology Osato, Kazuhiro; Mie University Graduate School of Medicine, Obstetrics and Gynecology Kamimoto, Yuki; Mie University Graduate School of Medicine, Obstetrics and Gynecology Tamaru, Satoshi; Mie University Hospital, Clinical Research Support Center Ogura, Toru; Mie University Hospital, Clinical Research Support Center Nishimura, Yuki; Mie University Hospital, Clinical Research Support Center Kodera, Mayumi; Mie University Hospital, Clinical Research Support Center Minamide, Chisato; Mie University Hospital, Clinical Research Support Center Nishikawa, Masakatsu; Mie University Hospital, Clinical Research Support Center Endoh, Masayuki; Osaka University Graduate School of Medicine, Obstetrics and Gynecology Kimura, Tadashi; Osaka University Graduate School of Medicine, Obstetrics and Gynecology Kotani, Tomomi; Nagoya University Graduate School of Medicine, Obstetrics and Gynecology Nakamura, Masamitsu; Showa University School of Medicine, Obstetrics and Gynecology Sekizawa, Akihiko; Showa University School of Medicine, Obstetrics and Gynecology Ikeda, Tomoaki; Mie University School of Medicine, Obstetrics and Gynecology
Primary Subject Heading:	Obstetrics and gynaecology

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Secondary Subject Heading:	Obstetrics and gynaecology, Research methods
Keywords:	Fetal growth restriction, Phosphodiesterase 5 inhibitor, Tadalafil, Phase II trial, Study protocol

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Manuscripts

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5 **Title: TADAFER II: Tadalafil treatment for fetal growth restriction - a study protocol for**
6 **a multicenter randomized controlled phase II trial.**
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10 Running head: Tadalafil for fetal growth restriction
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12 Takashi Umekawa,¹ Shintaro Maki,¹ Michiko Kubo,¹ Hiroaki Tanaka,¹ Masafumi Nii,¹ Kayo
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16 behalf of the TADAFER study group.
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45 **Disclosure**

46 The authors declare no conflict of interest.
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49 **Word count:** 4495 words.
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1 **Title: TADAFER II: Tadalafil treatment for fetal growth restriction - a study protocol for**
2 **a multicenter randomized controlled phase II trial.**

3
4 **ABSTRACT**

5 **Introduction:** There is no proven therapy to reverse or ameliorate fetal growth restriction
6 (FGR). Sildenafil, a selective phosphodiesterase 5 (PDE5) inhibitor, has been reported to
7 potentially have a therapeutic role in FGR, but this has not been established. Tadalafil is also a
8 selective PDE5 inhibitor. We have demonstrated efficacy for tadalafil on fetal growth in FGR
9 and the short-term outcomes and feasibility of tadalafil in FGR. Based on the hypothesis that
10 tadalafil will safely increase the likelihood of increased fetal growth in FGR, we have designed
11 this phase II study to prospectively evaluate the efficacy and safety of tadalafil in FGR.

12 **Methods and analysis:** This study is a multicenter randomized controlled phase II trial. A total
13 of 140 fetuses with FGR will be enrolled from medical centers in Japan. Fetuses will be
14 randomized to receive either the conventional management for FGR, according to the guidelines,
15 or a once daily treatment with 20 mg of tadalafil along with the conventional management, until
16 delivery. The primary endpoint is fetal growth velocity from the first day of the protocol-defined
17 treatment to birth (g/day), and is calculated using the following formula:

$$\text{Fetal growth velocity (g/day)} \\ = \frac{\text{Birthweight} - \text{Estimated fetal weight at the first day of the treatment [g]}}{\text{Days of the treatment [days]}}$$

18 To minimize bias in terms of fetal baseline conditions and timing of delivery, a fetal indication
19 for delivery is established in this study. The investigator will evaluate fetal baseline conditions
20 at enrollment and will decide the timing of delivery based on this fetal indication. Infants will be
21 followed up for development until 1.5 years of age.

22 **Ethics and dissemination:** This study was approved by the Institutional Review Board of Mie
23 University Hospital and each participating institution. Our findings will be widely disseminated
24 through peer-reviewed publications.

25 **Trial registration:** UMIN000023778.

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1 **Strengths and limitations of this study**

- 2 • This is a multicenter randomized controlled phase II trial to prospectively evaluate the
3 efficacy and safety of tadalafil treatment in fetuses with fetal growth restriction (FGR), for
4 which there is no proven therapy.
- 5 • This trial will include the participation of major medical centers providing treatment for
6 fetuses with FGR according to the guidelines for obstetrical practice in Japan.
- 7 • To minimize bias in terms of fetal baseline conditions and timing of delivery, a fetal
8 indication for delivery is established in this study on the basis of the results from a
9 multicenter survey in Japan.
- 10 • The possible limitation is related to open-label trial features, in which enrolled participants
11 receive either the conventional management for FGR according to the guidelines for
12 obstetrical practice in Japan, or a once daily treatment with 20 mg of tadalafil added to the
13 conventional management.

1 INTRODUCTION

2 Neonatal intensive care has improved over the past few decades, and morbidity among
3 infants, including those who are premature, continues to decline. Premature infants with
4 intrauterine growth restriction, however, still have high mortality and morbidity. The multicenter
5 survey[1] of very low birth weight (VLBW) infants in Japan conducted by Kusuda and Ikeda *et*
6 *al.* revealed that mortality in neonatal intensive care units (NICU), of small gestational age
7 (SGA) infants born before 30 weeks gestation, was significantly higher than that of appropriate
8 for gestational age (AGA) infants (unpublished data). To prevent fetal growth restriction (FGR),
9 nutritional and dietary supplementation, bed rest, and aspirin therapy have been investigated,
10 but there is insufficient evidence for the routine indication of any of these treatments.[2] There
11 is also no proven therapy to reverse or ameliorate established FGR.[3]

12 Increases in uteroplacental blood flow during pregnancy via angiogenesis and
13 vasodilation contribute to adequate fetal growth. Vasodilation in the uteroplacental unit is
14 considered to be due to the production and local release of nitric oxide (NO), which stimulates
15 cyclic guanosine monophosphate (cGMP) production.[4] cGMP is inactivated mainly by
16 phosphodiesterases (PDE), and the predominant PDE isoform present in the vascular smooth
17 muscle is PDE5. Because inhibitors of PDE5, which is a cGMP-specific PDE, exert their
18 pharmacological action by dilating arteries and increasing blood flow, as proven in erectile
19 dysfunction and pulmonary hypertension, recent studies have suggested a potential therapeutic
20 role for PDE5 inhibitors in treating FGR.[5] Sildenafil, a selective PDE5 inhibitor, has been
21 shown to improve endothelial function in myometrial small arteries removed from women with
22 pre-eclampsia and FGR.[6, 7] However, although sildenafil has been reported to affect maternal
23 hypertension, it has not been shown to affect FGR in studies in FGR model rats induced by
24 L-NG-nitroarginine methyl ester (L-NAME) treatment. In these studies, FGR was not affected
25 by sildenafil except in one report, by Baijnath *et al.*[8-11] Baijnath *et al.* demonstrated that
26 L-NAME-induced FGR was improved by sildenafil treatment from 4 days postcoitum (d.p.c.) to
27 8 d.p.c. but not from 8 d.p.c. to 14 d.p.c.[10] Chorioallantoic attachment occurs at 8 d.p.c., and
28 the mature circulatory pattern of maternal blood through the placenta is established by 10 d.p.c.
29 in mouse placenta.[12, 13] In considering the development of fetoplacental circulation in
30 rodents, the effect of sildenafil on fetal growth associated with placental blood flow via an
31 NO-dependent pathway was not manifested. In a clinical study, it was reported that sildenafil
32 was associated with increased fetal abdominal circumference (AC) growth velocity in severe

1 early-onset FGR, but the authors did not report on fetal growth velocity and birth weight.[14]
2 Recently, the STRIDER UK group has found no evidence of a beneficial effect of sildenafil on
3 survival or short-term neonatal outcomes.[15]

4 Tadalafil is also a selective PDE5 inhibitor and has a longer half-life and a more rapid
5 onset of action than sildenafil.[5] Tadalafil has been used to treat pulmonary hypertension in
6 pregnant women and the Food and Drug Administration in the United States has rated tadalafil
7 as pregnancy category B.[16] Ladouceur *et al.* reported pregnancy outcomes in patients with
8 pulmonary arterial hypertension associated with congenital heart disease treated with tadalafil.
9 They did not describe adverse effects associated with tadalafil.[17] Doimon *et al.* also reported
10 no side effects of tadalafil on mothers or offsprings in cases with pulmonary arterial
11 hypertension treated with tadalafil.[18] When taking sildenafil with a high-fat meal, the time to
12 maximum plasma concentration increases and the peak plasma concentration falls.[19] In
13 contrast, Fogue *et al.* reported that food intake had a negligible effect on the bioavailability of
14 tadalafil, and also reported that there was no clinically meaningful effect of gender on tadalafil
15 pharmacokinetics.[20] Our animal experiments demonstrated that tadalafil treatment dilates the
16 maternal blood sinuses in the placenta, which leads to increased placental growth factor (PlGF)
17 production, and contributes to facilitating fetal growth.[21] Because tadalafil treatment was
18 started after blood spaces in the placenta were narrowed by L-NAME treatment and elevated
19 urinary excretion of cGMP in these animal experiments, we can safely presume that tadalafil
20 treatment contributes to facilitating fetal growth in the context of the mechanisms associated
21 with NO signaling. In addition, we retrospectively analyzed 11 Japanese singleton pregnant
22 women with FGR who received tadalafil along with conventional management for FGR at Mie
23 University Hospital from July 2015 to February 2016 (tadalafil group).[22] These women were
24 matched for maternal age, parity, gestational age (GA), and estimated fetal weight at enrollment
25 with 14 singleton pregnant women who had received only the conventional management for
26 FGR in 2014 (conventional management group). The conventional management for FGR was
27 performed according to the guidelines for obstetric practice in Japan.[23] This retrospective
28 study showed that both fetal growth velocity from enrollment to birth and birth weight were
29 significantly higher in the tadalafil group than in the conventional management group. Moreover,
30 the prevalence of respiratory distress syndrome (RDS) was significantly lower in the tadalafil
31 group than in the conventional management group. After the retrospective study, we conducted
32 a phase I clinical trial to ensure the safety of tadalafil treatment for FGR.[24] There were no

1 serious maternal adverse events for daily tadalafil doses of 10 mg, 20 mg, and 40 mg. More
2 patients who were administered 40 mg tadalafil daily experienced mild adverse events than
3 those administered 10 mg or 20 mg tadalafil daily. In regards to fetal adverse events,
4 intrauterine fetal death occurred in one case. In this case, the pregnant woman was prescribed 40
5 mg tadalafil daily and fetal growth had been progressing at a rate of 22 g/day. At 36 weeks
6 gestation, fetal movement suddenly ceased and a diagnosis of intrauterine fetal death was made.
7 Thereafter, the fetus was delivered vaginally, and velamentous insertion of the umbilical cord
8 was identified. Immediately, the safety evaluation committee investigated the incident's
9 relationship to tadalafil. This committee analyzed the case and concluded that the intrauterine
10 fetal death was due to velamentous insertion of the umbilical cord.[25] We concluded that
11 tadalafil treatment was feasible in pregnant women with FGR.[24]

12 Based on the above, we have hypothesized that tadalafil therapy will safely increase the
13 likelihood of increased fetal growth in fetuses with FGR and have designed this multicenter
14 randomized controlled phase II study to establish evidence for fetal therapy with tadalafil. This
15 study, funded by the Japan Agency for Medical Research and Development (AMED), will
16 prospectively evaluate the safety and efficacy of tadalafil in FGR with the participation of major
17 medical centers providing treatment for fetuses with FGR according to the guidelines for
18 obstetrical practice in Japan.

19 **METHODS**

20 **Study design**

21 This study is a multicenter randomized controlled phase II trial.

22 **Study period**

23 The planned study period is from the date of ethics approval to February 2021. The
24 Patient Registration Period will last until December 2018. The children's outcome will be
25 followed up for 1.5 years after birth. Data collected by the end of the Neonatal Evaluation
26 Period will be subjected to statistical analysis.

27 Patient Registration Period: date of ethics approval to December 2018.

28 Children's Outcome Follow-up Period: 1.5 years after the last birth.

29 **Patient selection**

30 Inclusion criteria are as follows: (1) Pregnant women ≥ 20 years; (2) Estimated fetal
31 weight (EFW) less than 1.5 standard deviations of the mean EFW for GA; (3) GA between 20 +

0 and 33 + 6 weeks; (4) The expected date of confinement is determined using the criteria of the guidelines for obstetrical practice in Japan (2014); (5) Singleton pregnancy; and (6) Signed written informed consent.

Exclusion criteria are as follows; (1) A result from the antepartum fetal tests, done at enrollment, which indicates that delivery should be attempted*; (2) A history of allergy to tadalafil; (3) Concurrent medications that interact adversely with tadalafil; (4) Contraindication of tadalafil treatment due to renal disease; (5) Contraindication of tadalafil treatment due to liver disease; (6) Contraindication of tadalafil treatment due to uncontrolled arrhythmia, hypertension (BP >170/100 mmHg), and hypotension (BP <80/40 mmHg); (7) Fetus with suspected chromosomal disorder and/or multiple congenital anomalies; (8) Contraindication of tadalafil treatment due to retinitis pigmentosa, coagulation defect, active gastric and/or intestinal ulcer, or venous obstructive disease; and (9) The investigator decides that entry is inappropriate**.

* To minimize bias in terms of fetal baseline condition at enrollment, a fetal indication for delivery is established on the basis of the results from the multicenter survey of VLBW infants in Japan using a network database, in which the 82 level III perinatal centers were registered. The survey data included infant survival rate in the NICU, categorized by birth weight and gestational week at birth (Figure 1).[1] The infant survival rate data acquired from the survey were preprocessed with the moving average method and divided into three groups. The first group was defined as “Zone 1” where the infant survival rate in the NICU was less than 60%. The second group was defined as “Zone 2” where the infant survival rate in the NICU ranged from 60 to 95%. The third group was defined as “Zone 3” where the infant survival rate in the NICU was 95% or higher. All patients in our study will undergo antepartum fetal tests consisting of the evaluation of fetal well-being by ultrasonography, including Doppler imaging of umbilical arterial blood flow, non-stress test, contraction stress test, and biophysical profile scoring depending on GA, to evaluate possible pregnancy termination by the investigator at enrollment (Table 1). [23, 25, 26]

Table 1. A fetal indication for delivery in the TADAFER II study. [23, 25, 26]

Infant survival rate in the NICU (See Figure 1)	
Zone 1	Decide timing of delivery depending on available therapeutic measures at the NICU in each institute.
Zone 2	Consider delivery if at least one of three findings is made, but place give high priority on the determination by the investigators. <ol style="list-style-type: none"> 1. Reversed umbilical artery blood flow during diastole. 2. Score less than 4 on the fetal biophysical profile score. 3. Feat heart rate patterns in the orange or red category for more than 30 minutes. [26]
Zone 3	Consider delivery if at least one of five findings is made, but place give high priority on the determination by the investigators. <ol style="list-style-type: none"> 1. Reversed or absent umbilical artery blood flow during diastole.

	2.	Score less than 4 on the fetal biophysical profile score. (Score less than 6 on the fetal biophysical profile score if oligohydramnios is present.)
	3.	Feat heart rate patterns in the orange or red category for more than 30 minutes. [26]
	4.	Positive contraction stress test.
	5.	Impaired fetal head circumference growth for more than 2 weeks.

**Regarding exclusion criteria No.9 “The investigator decides that entry is inappropriate”, this study excludes mothers with mental or psychiatric problems, since poor judgment capabilities that are often associated with such conditions may not be compatible with Inclusion Criterion No. 6 “Signed written informed consent”.

Registration

The study protocol defines all of the procedures and schedules that the investigator must abide by to complete this clinical study, including patient selection and registration, fetal treatment of FGR, and follow-up (Figure 2). Patients that satisfy all inclusion criteria and do not meet any of the exclusion criteria will be eligible for inclusion in the study. Individual study sites will be responsible for guiding potential participants through the informed consent process, including patients who have been referred to them for treatment purposes. The investigator will enter an eligible patient’s information into the Eligibility Confirmation Form on the website of this clinical trial (the Clinical Trial Data Management System: Japanese-only website). The data management system will check the contents of the form before registering the patient. For patients who meet all inclusion criteria without violating any of the exclusion criteria listed above, the data management system will register and allocate them to the two arms in an allocation ratio of 1:1, one group receiving the conventional management of FGR according to the guidelines for obstetrical practice in Japan,[23] and the other receiving once-daily treatment with 20 mg tadalafil added to conventional management after adjustment for study sites and GA (<28 or ≥28 weeks of gestation). The investigators are blinded to the allocation algorithm. Enrolled participants will receive fetal therapy within 7 days of registration. The investigator will enter the patients’ data into the Case Report Form on the website of this clinical trial (the Clinical Trial Data Management System).

Fetal Treatment Protocol

The investigator will provide the fetal therapy as described below.

Arm A: the conventional management of FGR according to the guidelines for obstetrical practice in Japan.[23] Briefly, the conventional management of FGR consists of evaluation of fetal well-being on ultrasonography, including Doppler imaging of umbilical arterial blood flow, non-stress test, contraction stress test, and biophysical profile scoring depending on GA to evaluate possible pregnancy termination.

1 *Arm B*: once-daily treatment with 20 mg tadalafil added to the conventional management until
2 delivery.

3 The investigators are blinded to the allocation algorithm. Enrolled participants will receive fetal
4 therapy within 7 days of registration.

5 6 **Endpoints**

7 **(1) Primary endpoint**

8 Fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day).

9 The primary endpoint is fetal growth velocity from the first day of the protocol-defined
10 treatment to birth (g/day), and is calculated using the following formula:

$$11 \quad \text{Fetal growth velocity (g/day)} \\ 12 \quad = \frac{\text{Birthweight} - \text{EFW at the first day of the treatment [g]}}{\text{Days of the treatment [days]}}$$

13 **Rationale for the primary endpoint**

14 Our primary hypothesis is that tadalafil therapy will increase the likelihood of increased
15 fetal growth velocity in fetuses with FGR. Taking into account the results of our retrospective
16 study demonstrating that tadalafil treatment increased fetal growth velocity (g/day), which was
17 the primary outcome of the retrospective study, and decreased the incidence of RDS, an
18 improvement in fetal growth velocity from the first day of the protocol-defined treatment to
19 birth (g/day) is an important indicator of the therapeutic benefits for fetuses with FGR.[22]

20 **(2) Secondary endpoints**

21 1) Completion rate of the treatment regimen.

22 Completion rate of the treatment regimen is defined as the percentage of enrolled patients who
23 receive the protocol-defined treatment for more than 7 days.

24 2) Efficacy endpoints.

25 i) Estimated fetal weight (g).

26 Estimated fetal weight (EFW) is calculated using the following formula:[27]

$$27 \quad \text{EFW (g)} = 1.07 \times (\text{biparietal diameter: BPD})^3 + 0.3 \\ 28 \quad \quad \quad \times (\text{abdominal circumference: AC})^2 \times (\text{femur length: FL})$$

29 ii) Fetal growth velocity in the two weeks after the protocol-defined treatment and in the two
30 weeks after one week of the protocol-defined treatment (g/day).

31 Fetal growth velocity in the two weeks after the protocol-defined treatment (g/day) is calculated
32 using the following formula:

$$33 \quad \text{Fetal growth velocity in the two weeks after the protocol – defined treatment (g/day)} \\ 34 \quad = \frac{(\text{EFW two weeks after the treatment} - \text{EFW at the first day of the treatment [g]})}{14 \text{ [days]}}$$

1 and fetal growth velocity in the two weeks after one week of the protocol-defined treatment
 2 (g/day) is calculated using the following formula:

$$\begin{aligned} & \text{Fetal growth velocity in the two weeks after one week of the treatment (g/day)} \\ & = \frac{(\text{EFW three weeks after the treatment} - \text{EFW one week after the treatment [g]})}{14 \text{ [days]}} \end{aligned}$$

3
 4 iii) Fetal growth rate in the two weeks after the protocol-defined treatment and from
 5 the first day of the protocol-defined treatment to birth (%/day).

6 Fetal growth rate in the two weeks after the protocol-defined treatment (%/day) is calculated
 7 using the following formula:

$$\begin{aligned} & \text{Fetal growth rate in the two weeks after the protocol-defined treatment (%/day)} \\ & = \frac{\text{EFW two weeks after the treatment} - \text{EFW at the first day of the treatment [g]} \times 100}{14 \text{ [days]} \times \text{EFW at the first day of the treatment [g]}} \end{aligned}$$

9 and fetal growth rate from the first day of the protocol-defined treatment to birth (%/day) is
 10 calculated using the following formula:

$$\begin{aligned} & \text{Fetal growth rate from the first day of the protocol-defined treatment to birth (%/day)} \\ & = \frac{\text{Birthweight} - \text{EFW at the first day of the treatment [g]} \times 100}{\text{Days of the treatment [days]} \times \text{EFW at the first day of the treatment [g]}} \end{aligned}$$

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 12 iv) Fetal head circumference (cm).

13 The fetal head circumference was measured at the plane of the third ventricle with the thalamus
 14 in the central portion and the cavum septi pellucidi visible in the anterior portion.

15 v) Doppler imaging of umbilical arterial blood flow.

16 Umbilical arterial blood flow was examined by Doppler ultrasound according to the Society for
 17 Maternal-Fetal Medicine (SMFM) Clinical Guidelines.[28]

18 vi) Deepest amniotic fluid pocket (cm).

19 The deepest amniotic fluid pocket was measured by transabdominal ultrasonography.

20 iv) Fetal head circumference, vi) deepest amniotic fluid pocket, and v) doppler imaging of
 21 umbilical arterial blood flow are evaluated according to the flow chart as shown below.

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1 **Fetal head circumference, deepest amniotic fluid pocket, and doppler imaging of umbilical**
 2 **arterial blood flow evaluation flow chart.**

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5		
	Day of enrollment	1 week after the enrollment	2 weeks after the enrollment	3 weeks after the enrollment	4 weeks after the enrollment	Every two weeks before 36 weeks of GA after visit 5	Every one weeks at or after 37 weeks of GA
Fetal head circumference	•	•	•	•	•	•	•
Deepest amniotic fluid pocket	•	•	•	•	•	•	•
Doppler imaging of umbilical arterial blood flow	•	•	•	•	•	•	•

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4 vii) Prolongation of GA at birth (days).

5 Prolongation of GA at birth is defined as days from the first day of the protocol-defined
 6 treatment to birth.

7 viii) Birth weight (g).

8 Birth weight is defined as the weight of the infant at birth.

9 ix) GA at birth.

10 GA at birth is defined as the gestational age at birth.

11 x) Apgar score.

12 The Apgar score consists of an evaluation of five factors: heart rate, respiratory effort, muscle
 13 tone, responsiveness, and color at one minute and five minutes after birth.

14 xi) Umbilical artery pH and base excess values.

15 Umbilical artery pH and base excess is measured at delivery.

16 xii) Incidence rate of pre-eclampsia.

17 Incidence rate of pre-eclampsia is defined as the percentage of enrolled patients who develop
 18 pre-eclampsia after the protocol-defined treatment.

19 xiii) Pediatric developmental assessment until 1.5 years of age.

20 Pediatric developmental assessment includes physiological and neurological developmental
 21 assessment, and infant complications including cerebral palsy, epilepsy, and death.

22 3) Safety endpoints

23 i) Incidence rate of obstetric complications.

24 Incidence rate of obstetric complications including hypertensive disorders of pregnancy (HDP)
 25 is defined as the percentage of enrolled patients who develop obstetric complications after the
 26 protocol-defined treatment.

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5 ii) Perinatal mortality.

6 Perinatal mortality is defined to include stillbirths (occurring after 22 weeks of gestation) and
7 neonatal deaths (occurring up to 7 days after birth).

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9 iii) Neonatal mortality.

10 Neonatal mortality is defined as neonatal deaths occurring up to 28 days after birth.
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13 **Stopping Criteria**

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15 The investigator must discontinue the protocol-defined treatment when certain events
16 prevent continuation of the protocol treatment. These events include the following:

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18 1. The mother has withdrawn her consent to study participation.

19 2. Certain events prevent continuation of the protocol treatment, which include the following:

20 a) A serious adverse drug reaction to tadalafil has developed.

21 b) The investigator's decision to prioritize other management including termination of the
22 pregnancy instead of continuation of the protocol-defined treatment.

23 c) The investigator's decision that it is inappropriate to continue with the protocol treatment.

24 d) The mother's poor compliance or discontinuation of the protocol treatment.
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29 **Criteria for Delivery**

30 In this study, to minimize bias in terms of the timing of delivery, a fetal indication for
31 delivery is established on the basis of the results from the multicenter survey of VLBW infants
32 in Japan using a network database (Figure 1 and Table 1). After registration, all patients will
33 receive the conventional management of FGR according to the guidelines for obstetrical
34 practice in Japan regardless of the treatment arm.[23] Briefly, the conventional management of
35 FGR consists of the evaluation of fetal well-being on ultrasonography, including Doppler
36 imaging of umbilical arterial blood flow, non-stress test, contraction stress test, and biophysical
37 profile scoring depending on GA, to evaluate possible pregnancy termination. The investigator
38 will evaluate the fetal condition and decide timing of delivery referring to Table 1. For other
39 complications such as preterm labor, rupture of the membranes, and hypertensive disorder of
40 pregnancy, the investigator will follow guidelines for obstetric practice in Japan.[23] The
41 investigator must provide a report that explains the reason for termination of the pregnancy on
42 the website of this clinical trial (the Clinical Trial Data Management System).
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50 **Monitoring Safety during the Fetal Therapy**

51 The investigator must pay close attention to the safety of not only the fetus but also the
52 mother. As shown in the study schedule, the protocol-defined assessments include evaluation of
53 maternal blood pressure and pulse rate, maternal blood and urine tests (blood
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1 fibrinogen and anti-thrombin III levels, liver and renal function tests, serum electrolyte levels,
2 and qualitative urine protein excretion), maternal serum placental growth factor (PIGF) and
3 soluble fms-like tyrosine kinase receptor (sFLT-1) levels. Other assessments include adverse
4 events assessed by medical consultation, and antepartum fetal tests consisting of
5 ultrasonography including Doppler imaging of blood flow (umbilical artery, middle cerebral
6 artery, and uterine artery), non-stress test, contraction stress test, and biophysical profile scoring
7 depending on GA. The investigator will enter patients' safety data into the Case Report Form on
8 the website of this clinical trial (the Clinical Trial Data Management System).

9 10 **Safety Evaluation Committee**

11 The Safety Evaluation Committee is responsible for the overall safety of this clinical
12 study. To ensure the safety of the protocol-defined treatment, the Safety Evaluation Committee
13 will review the adverse events of tadalafil treatment. If a serious adverse event develops, the
14 investigator will provide the Secretariat with the necessary information within 24 hours of its
15 onset, according to the predetermined procedure. The Secretariat then will forward the obtained
16 information without delay to the Safety Evaluation Committee for review. The Safety
17 Evaluation Committee will notify the investigator of the review results. If the adverse event is
18 definitely or probably related to tadalafil treatment, the Ethics Committee in Mie University
19 Hospital or each institute will consider possible termination of this clinical study. Special
20 attention must be paid to the reporting requirements stipulated in the Ethical Guidelines for
21 Clinical Studies (Ministry of Health, Labor, and Welfare in Japan, 2017). Infants will be
22 followed up and evaluated for physiological and neurological development until 1.5 years of
23 age.

24 25 **Sample size**

26 140 fetuses and their mothers.

27 **Rationale for the Target Sample Size**

28 Table 2 shows the summary of the distribution of fetal growth velocity from enrollment to
29 birth in our retrospective study.[22] We estimate that the distribution of fetal growth velocity of
30 this prospective phase II trial will be similar to that of our retrospective study. When the results
31 of our prospective study are analyzed by Wilcoxon Rank Sum Test and group comparisons, with
32 an α of 0.05, two sided, we will have 90% power to detect a difference if we randomize 62
33 women per group. Allowing for a 10% drop-out rate, the total sample size required is 140
34 women.

Table 2. The distribution of fetal growth velocity from enrollment to birth in the retrospective study conducted at Mie University Hospital.

Fetal growth velocity (g/day)	<5	≥5 to <10	≥10 to <15	≥15 to <20	≥20 to <25	≥25
The conventional management group (%)	5.3	10.5	21.1	47.3	15.8	0
The tadalafil group (%)	0	8.3	8.3	50.0	16.7	16.7

Statistical analysis

Analysis is done on all randomized fetuses who receive the protocol-defined treatment at least once, as the full analysis set. Analysis per protocol set (i.e., removing patients who do not meet the inclusion and exclusion criteria) is done as a secondary analysis population for sensitivity analysis. All outcome measures are presented as summaries of descriptive statistics (mean [SD] or median [minimum, maximum, and interquartile range] for continuous measures, and the numbers and proportions for ordinal and dichotomous measures). Descriptive statistics for the primary endpoint, i.e. fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day), are analyzed for each treatment arm by the Wilcoxon Rank Sum Test and group comparisons. All analyses are performed according to a pre-specified statistical analysis plan. The Data Coordinating Center in Mie University Hospital supports the data management, statistical analysis, and reporting of the study.

Ethics and dissemination

This study was approved by the Institutional Review Board of Mie University Hospital on August 25th, 2016 (No.3041) prior to patient enrollment. The study protocol was also approved by each institutional review board of all participating institutions. This study complies with the Helsinki Declaration. Written informed consent will be obtained from all mothers of fetuses before they are recruited. This trial has been registered in the UMIN Clinical Trials Registry as UMIN000023778 (https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno=R000027132). Our findings will be widely disseminated through conference presentations and peer-reviewed publications.

Participating institutions

Mie University, Showa University, Osaka University, Nagoya University, Mie Chuo Medical Center, Municipal Yokkaichi hospital, Ise Red Cross Hospital, St. Marianna University, Juntendo University, the Jikei University, Toho University, Yokohama City University Medical Center, Kanagawa Children's Medical Center, Ehime University, Hamamatsu University School of Medicine, Osaka Medical College, Niigata University, Showa University Northern Yokohama Hospital, Showa University Koto Toyosu Hospital, Gifu University, University of the Ryukyu,

1 Shiga University, Shinshu University, Ehime Prefectural Central Hospital, Akita University,
2 Tokyo Metropolitan Bokutoh Hospital, Kyorin University, Tokyo Metropolitan Tama Medical
3 Center, Kuwana East Medical Center, Kanazawa University, Nagasaki Medical Center,
4 University of Toyama, Yamaguchi University, Toyota Memorial Hospital, Kainan Hospital,
5 Dokkyo Medical University, Saga Hospital, Kyoto Prefectural University, Toyama Central
6 Prefectural Hospital, Sapporo City General Hospital, Kagoshima University, Mie Prefectural
7 General Medical Center, Kyoto University, Sakakibara Heart Institute, and University of Fukui.

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9 The original protocol is available in *the supplementary file*.

10 11 **DISCUSSION**

12 This protocol has been already approved by the Institutional Review Board of Mie
13 University Hospital and 39 institutions in Japan. Fetuses with FGR will be enrolled from these
14 institutions. Because fetal growth velocity from the first day of the treatment to birth has been
15 defined as the primary endpoint and fetuses will be randomly assigned in an open-label design,
16 timing of delivery should be made on the basis of similar criteria as much as possible. This
17 study is the first nation-wide intervention study in the field of obstetrics in Japan. We selected
18 an open-label study design with a strict fetal management algorithm on the basis of the results
19 from the multicenter Japanese survey instead of a placebo-controlled design because of
20 operational challenges including low acceptability by pregnant women in Japan. Each
21 participating medical center can provide treatment for fetuses with FGR by board certified
22 members of the Japan Society of Obstetrics and Gynecology, and the investigator will be able to
23 optimally decide timing of delivery according to the guidelines for obstetrical practice in
24 Japan.[23] To make more accurate decisions, a fetal indication for delivery is established in this
25 study on the basis of the results from the multicenter survey in Japan, in which 82 level III
26 perinatal centers, including 8 sites participating in this study, were registered (Table 1).[1] The
27 fetal indication for delivery is divided into three groups depending on infant survival rate in the
28 NICU. Because all patients will undergo antepartum fetal tests consisting of evaluation of fetal
29 well-being by ultrasonography, including Doppler imaging of umbilical arterial blood flow,
30 non-stress test, contraction stress test, and biophysical profile scoring depending on GA
31 according to the Japanese guidelines, the investigator will easily refer to this indication when
32 deciding timing of delivery. This indication will be used to evaluate fetal baseline condition at
33 enrollment as well. We believe that this approach could take advantage of strengths and
34 minimize the possible limitations related to open-label trial features.

35 We retrospectively compared the effect of tadalafil in patients with FGR and
36 demonstrated that both fetal growth velocity from enrollment to birth and birth weight were

1 significantly higher in the tadalafil group than in the conventional management group. The
2 required sample size of this prospective study was estimated based on the results of the
3 retrospective study that used the same primary outcome measure. Since patients with FGR were
4 enrolled in the retrospective study under similar criteria to those in this study, we think that it is
5 reasonable to use the results of the retrospective study for the estimation of sample size.
6

7 **Contributors:** T.U., S.M., M.K., H.T., M.N., K.T., K.O., Y.K., M.E., T. Kimura, T. Kotani, M.N.,
8 A.S., and T.I.: conception of the study. T.U.: writing of the manuscript. S.T., Y.N., M.K., C.M.,
9 and M.N.: providing the biostatistical study design. T.O.: statistical analysis. T. I.: principal
10 Investigator of this trial and the grant holder. All authors have read and approved the final
11 manuscript.
12

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16

17 **Competing interests:** None declared.
18

19 **Ethics approval:** The Institutional Review Board of of Mie University Hospital in Augst 25th,
20 2016 (No.3041).
21

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23 expenditures of our funds, and we are not prepared for data sharing at present. In the future, if
24 the chief researcher receives requests, we will prepare for data sharing to the extent permitted by
25 the Japanese ethics guidelines.
26

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29 Member of the Japan Society of Obstetrics and Gynecology) for their contribution as members
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31 (Department of Pediatrics, Anesthesiology, and Critical Care Medicine, Mie University
32 Graduate School of Medicine) for his advice on the protocol of
33 pediatric developmental assessment.
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REFERENCES

- 1 Kusuda S, Fujimura M, Sakuma I, *et al.* Morbidity and mortality of infants with very
2 low birth weight in Japan: center variation. *Pediatrics* 2006;**118**:e1130–e1138.
- 3 American College of Obstetricians and Gynecologists. ACOG Practice bulletin no. 134:
4 fetal growth restriction. *Obstet Gynecol* 2013;**121**:1122–33.
- 5 Hui L, Challis D. Diagnosis and management of fetal growth restriction: the role of fetal
6 therapy. *Best Pract Res Clin Obstet Gynaecol* 2008;**22**:139–58.
- 7 Coppage KH, Sun X, Baker RS, *et al.* Expression of phosphodiesterase 5 in maternal
8 and fetal sheep. *Am J Obstet Gynecol* 2005;**193**:1005–10.
- 9 Rotella DP. Phosphodiesterase 5 inhibitors: current status and potential applications.
10 *Nat Rev Drug Discov* 2002;**1**:674–82.
- 11 Wareing M, Myers JE, O'Hara M, *et al.* Effects of a phosphodiesterase-5 (PDE5)
12 inhibitor on endothelium-dependent relaxation of myometrial small arteries. *Am J Obstet Gynecol*
13 2004;**190**:1283–90.
- 14 Wareing M, Myers JE, O'Hara M, *et al.* Sildenafil citrate (Viagra) enhances
15 vasodilatation in fetal growth restriction. *J Clin Endocrinol Metab* 2005;**90**:2550–5.
- 16 Herraiz S, Pellicer B, Serra V, *et al.* Sildenafil citrate improves perinatal outcome in
17 fetuses from pre-eclamptic rats. *BJOG Int J Obstet Gynaecol* 2012;**119**:1394–402.
- 18 Ramesar SV, Mackraj I, Gathiram P, *et al.* Sildenafil citrate improves fetal outcomes in
19 pregnant, L-NAME treated, Sprague-Dawley rats. *Eur J Obstet Gynecol Reprod Biol*
20 2010;**149**:22–6.
- 21 Baijnath S, Soobryan N, Mackraj I, *et al.* The optimization of a chronic nitric oxide
22 synthase (NOS) inhibition model of pre-eclampsia by evaluating physiological changes. *Eur J*
23 *Obstet Gynecol Reprod Biol* 2014;**182**:71–5.
- 24 Nassar AH, Masrouha KZ, Itani H, *et al.* Effects of sildenafil in N^ω-nitro-L-arginine
25 methyl ester-induced intrauterine growth restriction in a rat model. *Am J Perinatol* 2012;**29**:429–
26 34.
- 27 Cross JC, Hemberger M, Lu Y, *et al.* Trophoblast functions, angiogenesis and
28 remodeling of the maternal vasculature in the placenta. *Mol Cell Endocrinol* 2002;**187**:207–12.
- 29 Watson ED, Cross JC. Development of structures and transport functions in the mouse
30 placenta. *Physiol Bethesda Md* 2005;**20**:180–93.
- 31 von Dadelszen P, Dwinnell S, Magee LA, *et al.* Sildenafil citrate therapy for severe
32 early-onset intrauterine growth restriction. *BJOG Int J Obstet Gynaecol* 2011;**118**:624–8.
- 33 Sharp A, Comforth C, Jackson R, *et al.* OC01.05: STRIDER UK: a randomised
34 controlled trial of sildenafil therapy in dismal prognosis early-onset intrauterine growth
35 restriction. [abstract] *Ultrasound Obstet Gynecol* 2017;**50**:3.

- 1 16 Sahni S, Palkar AV, Rochelson BL, *et al.* Pregnancy and pulmonary arterial
2 hypertension: A clinical conundrum. *Pregnancy Hypertens Int J Womens Cardiovasc Health*
3 2015;**5**:157–164.
- 4 17 Ladouceur M, Benoit L, Radojevic J, *et al.* Pregnancy outcomes in patients with
5 pulmonary arterial hypertension associated with congenital heart disease. *Heart Br Card Soc*
6 2017;**103**:287–92.
- 7 18 Daimon A, Kamiya CA, Iwanaga N, *et al.* Management of pulmonary vasodilator
8 therapy in three pregnancies with pulmonary arterial hypertension. *J Obstet Gynaecol Res*
9 2017;**43**:935–938.
- 10 19 Wilkins MR, Wharton J, Grimminger F, *et al.* Phosphodiesterase inhibitors for the
11 treatment of pulmonary hypertension. *Eur Respir J* 2008;**32**:198–209.
- 12 20 Fogue ST, Patterson BE, Bedding AW, *et al.* Tadalafil pharmacokinetics in healthy
13 subjects. *Br J Clin Pharmacol* 2006;**61**:280–288.
- 14 21 Yoshikawa K, Umekawa T, Maki S, *et al.* Tadalafil Improves L-NG-Nitroarginine
15 Methyl Ester-Induced Preeclampsia With Fetal Growth Restriction-Like Symptoms in Pregnant
16 Mice. *Am J Hypertens* 2017;**31**:89–96.
- 17 22 Kubo M, Umekawa T, Maekawa Y, *et al.* Retrospective study of tadalafil for fetal
18 growth restriction: Impact on maternal and perinatal outcomes. *J Obstet Gynaecol Res*
19 2017;**43**:291–297.
- 20 23 Minakami H, Maeda T, Fujii T, *et al.* Guidelines for obstetrical practice in Japan: Japan
21 Society of Obstetrics and Gynecology (JSOG) and Japan Association of Obstetricians and
22 Gynecologists (JAOG) 2014 edition. *J Obstet Gynaecol Res* 2014;**40**:1469–1499.
- 23 24 Kubo M, Tanaka H, Maki S, *et al.* Safety and dose-finding trial of tadalafil administered
24 for fetal growth restriction: A phase-1 clinical study. *J Obstet Gynaecol Res* 2017;**43**:1159–1168.
- 25 25 Cunningham F, Leveno K, Bloom S, *et al.* *Williams Obstetrics, 24e.* Mcgraw-hill 2014.
- 26 26 Parer JT, Ikeda T. A framework for standardized management of intrapartum fetal heart
27 rate patterns. *Am J Obstet Gynecol* 2007;**197**:26–e1.
- 28 27 Shinozuka N. Fetal biometry and fetal weight estimation: JSUM standardization.
29 *Ultrasound Rev Obstet Gynecol* 2002;**2**:156–161.
- 30 28 Berkley E, Chauhan SP, Abuhamad A, *et al.* Doppler assessment of the fetus with
31 intrauterine growth restriction. *Am J Obstet Gynecol* 2012;**206**:300–308.

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5 **FIGURE LEGENDS**

6 **Figure 1. Infant survival rate in the NICU categorized by birth weight and gestational**
7 **week at birth (%).**

8 This figure is established on the basis of the results from the multicenter survey of VLBW
9 infants in Japan using a network database. The survey data included infant survival rates in the
10 NICU, categorized by birth weight and gestational week at birth.[1] The infant survival rate data
11 acquired from the survey were preprocessed with the moving average method and divided into
12 three groups. The first group was defined as “Zone 1” where the infant survival rate in the
13 NICU was less than 60% (highlighted by a red background). The second group was defined as
14 “Zone 2” where the infant survival rate in the NICU ranged from 60 to 95% (highlighted by a
15 yellow background). The third group was defined as “Zone 3” where the infant survival rate in
16 the NICU was 95% or higher (highlighted by a blue background).
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24 **Figure 2. Summary of the study design.**
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Birth weight (g)	1401-1500						96	99	100	99	99	99	99
	1301-1400						94	97	99	99	99	100	99
	1201-1300						98	99	99	99	99	99	100
	1101-1200					96	96	99	100	99	99	99	100
	1001-1100				96	98	98	98	99	99	98	98	98
	901-1000				95	96	97	97	98	99	99	98	97
	801-900			89	91	95	96	96	97	97	98	100	100
	701-800		84	86	90	93	93	95	99	98	94	95	100
	601-700		78	86	90	93	94	93	96	100	100		
	501-600	59	69	80	90	87	93	94	92	87			
	401-500	49	64	71	80	77	80	86	100	71			
	301-400	41	52	51	56	68	67	73	71				
	201-300	18	10	31	33	40							
		22	23	24	25	26	27	28	29	30	31	32	33
	Gestational week at birth												

Figure 1

Figure 1. Infant survival rate in the NICU categorized by birth weight and gestational week at birth (%).

173x177mm (300 x 300 DPI)

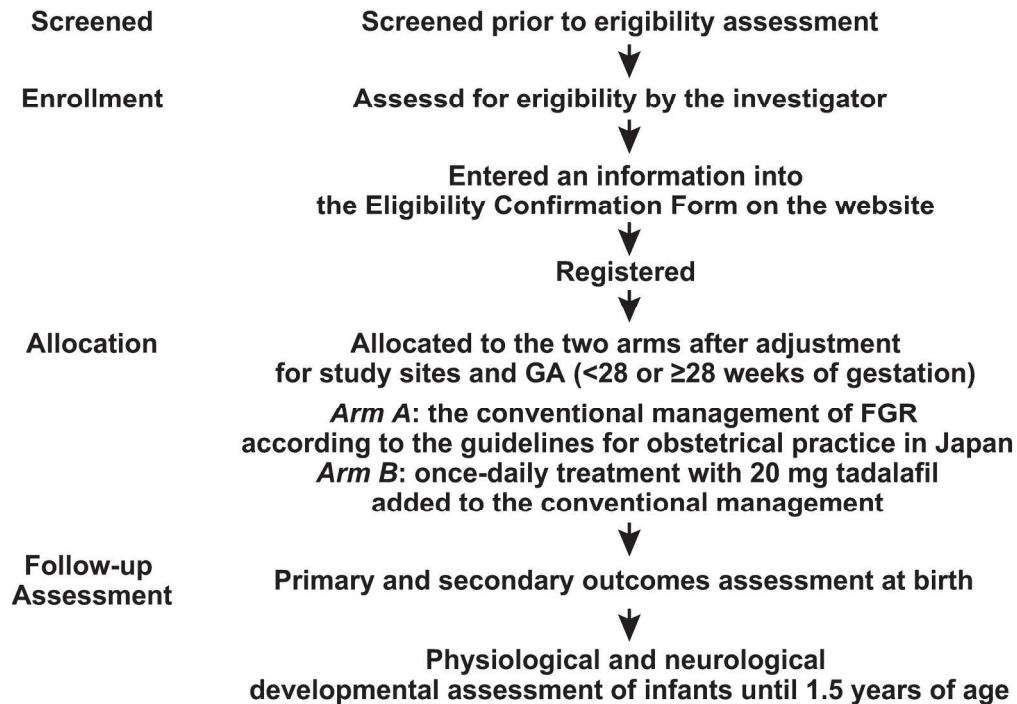


Figure 2

Figure 2. Summary of the study design.

212x193mm (300 x 300 DPI)

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Study Protocol

TADAFER II:

A multicenter phase II trial of the efficacy and safety of tadalafil in fetus with early-onset growth restriction.

Trial registration: UMIN Clinical Trials Registry UMIN000023778.

Version 1

Date 25-August-2016

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6 **Contents**

7 SYNOPSIS 3
8
9 1. VOLUNTARY PARTICIPATION AND WITHDRAWAL 5
10
11 2. BACKGROUND AND OBJECTIVES 5
12
13 3. RESEARCH ORGANIZATION AND PARTICIPATING INSTITUTIONS 8
14
15 4. STUDY SUBJECTS AND METHODS..... 10
16
17 5. TREATMENT AND COMPENSATION FOR STUDY-RELATED INJURY 19
18
19 6. STUDY PERIOD AND TARGET SAMPLE SIZE 20
20
21 7. OUTLINE OF THE STUDY PLAN..... 20
22
23 8. ANTICIPATED ADVERSE EVENTS..... 25
24
25 9. POTENTIAL BENEFITS AND RISKS 26
26
27 10. BURDEN OF COST..... 27
28
29 11. INTELLECTUAL PROPERTY RIGHTS 27
30
31 12. ETHICS 27
32
33 13. ADDITIONAL NOTES RELATED TO THE ACT ON THE PROTECTION OF
34 PERSONAL INFORMATION RELATED BY INDEPENDENT ADMINISTRATIVE
35 INSTITUTIONS..... 27
36
37 14. REFERENCE 29
38
39
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SYNOPSIS

1. Objectives

This multicenter randomized controlled phase II trial has been designed to prospectively evaluate the efficacy and safety of tadalafil treatment in fetus with fetal growth restriction (FGR).

2. Study eligibility

This study will include fetuses and their mothers who meet the following conditions:

- (1) Pregnant women ≥ 20 years.
- (2) Estimated fetal weight (EFW) less than 1.5 standard deviations of the mean EFW for gestational age.
- (3) Gestational age between 20 + 0 and 33 + 6 weeks.
- (4) The expected date of confinement is determined using the criteria of the guidelines for obstetrical practice in Japan (2014).¹
- (5) Singleton pregnancy.
- (6) Signed written informed consent.

3. Treatment

Fetuses with FGR will be randomized to receive either the conventional management of FGR according to the guidelines for obstetrical practice in Japan¹ or once-daily treatment with 20 mg tadalafil added to the conventional management until delivery.

4. Target sample size and duration of the study

Duration of the study: date of ethics approval to February 2021.

Target sample size: 140 singleton fetuses and their mothers.

5. Endpoints

- (1) Primary endpoint: fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day).
- (2) Secondary endpoints
 - 1) Completion rate of the treatment regimen
 - 2) Efficacy endpoints: estimated fetal weight (g), fetal growth velocity in the two weeks after the protocol-defined treatment (g/day), fetal growth velocity in the two weeks after one week of

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6 the protocol-defined treatment (g/day), fetal growth rate (%/day), fetal head circumference (cm),
7 Doppler imaging of umbilical arterial blood flow, deepest amniotic fluid pocket (cm),
8 prolongation of gestational age at birth (days), birth weight (g), gestational age (GA) at birth,
9 Apgar score, umbilical artery pH and base excess values, incidence rate of pre-eclampsia, and
10 pediatric developmental assessment until 1.5 years of age.
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14 3) Safety endpoints: incidence rate of obstetric complications, perinatal mortality, and neonatal
15 mortality.
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18 19 **6. Secretariats**

20
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1. VOLUNTARY PARTICIPATION AND WITHDRAWAL

Participation in this study is on a voluntary basis. Refusal to participate will incur no penalty or loss of benefits to which patients are otherwise entitled to. The subject may withdraw at any time without penalty.

2. BACKGROUD AND OBJECTIVES

Neonatal intensive care has improved over the past few decades, and morbidity among infants, including those who are premature, continues to decline. Premature infants with intrauterine growth restriction, however, still have high mortality and morbidity. The multicenter survey² of very low birth weight (VLBW) infants in Japan conducted by Kusuda and Ikeda *et al.* revealed that mortality in neonatal intensive care units (NICU), of small gestational age (SGA) infants born before 30 weeks gestation, was significantly higher than that of appropriate for gestational age (AGA) infants (unpublished data). To prevent fetal growth restriction (FGR), nutritional and dietary supplementation, bed rest, and aspirin therapy have been investigated, but there is insufficient evidence for the routine indication of any of these treatments.³ There is also no proven therapy to reverse or ameliorate established FGR.⁴

Increases in uteroplacental blood flow during pregnancy via angiogenesis and vasodilation contribute to adequate fetal growth. Vasodilation in the uteroplacental unit is considered to be due to the production and local release of nitric oxide (NO), which stimulates cyclic guanosine monophosphate (cGMP) production.⁵ cGMP is inactivated mainly by phosphodiesterases (PDE), and the predominant PDE isoform present in the vascular smooth muscle is PDE5. Because inhibitors of PDE5, which is a cGMP-specific PDE, exert their pharmacological action by dilating arteries and increasing blood flow, as proven in erectile dysfunction and pulmonary hypertension, recent studies have suggested a potential therapeutic role for PDE5 inhibitors in treating FGR.⁶ Sildenafil, a selective PDE5 inhibitor, has been shown to improve endothelial function in myometrial small arteries removed from women with pre-eclampsia and FGR.^{7,8} However, although sildenafil has been reported to affect maternal hypertension, it has not been shown to affect FGR in studies in FGR model rats induced by L-NG-nitroarginine methyl ester (L-NAME) treatment. In these studies, FGR was not affected by sildenafil except in one report, by Baijnath *et al.*^{9,10,11,12} Baijnath *et al.* demonstrated that L-NAME-induced FGR was improved by sildenafil treatment from 4 days postcoitum (d.p.c.) to

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6 8 d.p.c. but not from 8 d.p.c. to 14 d.p.c.¹¹ Chorioallantoic attachment occurs at 8 d.p.c., and the
7 mature circulatory pattern of maternal blood through the placenta is established by 10 d.p.c. in
8 mouse placenta.^{13,14} In considering the development of fetoplacental circulation in rodents, the
9 effect of sildenafil on fetal growth associated with placental blood flow via an NO-dependent
10 pathway was not manifested. In a clinical study, it was reported that sildenafil was associated
11 with increased fetal abdominal circumference (AC) growth velocity in severe early-onset FGR,
12 but the authors did not report on fetal growth velocity and birth weight.¹⁵ Recently, the
13 STRIDER UK group has found no evidence of a beneficial effect of sildenafil on survival or
14 short-term neonatal outcomes.¹⁶

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21 Tadalafil is also a selective PDE5 inhibitor and has a longer half-life and a more rapid
22 onset of action than sildenafil.⁶ Tadalafil has been used to treat pulmonary hypertension in
23 pregnant women and the Food and Drug Administration in the United States has rated tadalafil
24 as pregnancy category B.¹⁷ When taking sildenafil with a high-fat meal, the time to maximum
25 plasma concentration increases and the peak plasma concentration falls.¹⁸ In contrast, Forgue *et*
26 *al.* reported that food intake had a negligible effect on the bioavailability of tadalafil, and also
27 reported that there was no clinically meaningful effect of gender on tadalafil
28 pharmacokinetics.¹⁹ Our animal experiments demonstrated that tadalafil treatment dilates the
29 maternal blood sinuses in the placenta, which leads to increased placental growth factor (PIGF)
30 production, and contributes to facilitating fetal growth.²⁰ Because tadalafil treatment was started
31 after blood spaces in the placenta were narrowed by L-NAME treatment and elevated urinary
32 excretion of cGMP in these animal experiments, we can safely presume that tadalafil treatment
33 contributes to facilitating fetal growth in the context of the mechanisms associated with NO
34 signaling. In addition, we retrospectively analyzed 11 Japanese singleton pregnant women with
35 FGR who received tadalafil along with conventional management for FGR at Mie University
36 Hospital from July 2015 to February 2016 (tadalafil group).²¹ These women were matched for
37 maternal age, parity, gestational age (GA), and estimated fetal weight at enrollment with 14
38 singleton pregnant women who had received only the conventional management for FGR in
39 2014 (conventional management group). The conventional management for FGR was
40 performed according to the guidelines for obstetric practice in Japan.¹ This retrospective study
41 showed that both fetal growth velocity from enrollment to birth and birth weight were
42 significantly higher in the tadalafil group than in the conventional management group.
43 Moreover, the prevalence of respiratory distress syndrome (RDS) was significantly lower in the
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6 tadalafil group than in the conventional management group. After the retrospective study, we
7 conducted a phase I clinical trial to ensure the safety of tadalafil treatment for FGR.²² There
8 were no serious maternal adverse events for daily tadalafil doses of 10 mg, 20 mg, and 40 mg.
9 More patients who were administered 40 mg tadalafil daily experienced mild adverse events
10 than those administered 10 mg or 20 mg tadalafil daily. In regards to fetal adverse events,
11 intrauterine fetal death occurred in one case. In this case, the pregnant woman was prescribed 40
12 mg tadalafil daily and fetal growth had been progressing at a rate of 22 g/day. At 36 weeks
13 gestation, fetal movement suddenly ceased and a diagnosis of intrauterine fetal death was made.
14 Thereafter, the fetus was delivered vaginally, and velamentous insertion of the umbilical cord
15 was identified. Immediately, the safety evaluation committee investigated the incident's
16 relationship to tadalafil. This committee analyzed the case and concluded that the intrauterine
17 fetal death was due to velamentous insertion of the umbilical cord.²³ We concluded that tadalafil
18 treatment was feasible in pregnant women with FGR.²²

19 Based on the above, we have hypothesized that tadalafil therapy will safely increase the
20 likelihood of increased fetal growth in fetuses with FGR and have designed this multicenter
21 randomized controlled phase II study to establish evidence for fetal therapy with tadalafil. This
22 study, funded by the Japan Agency for Medical Research and Development (AMED), will
23 prospectively evaluate the safety and efficacy of tadalafil in FGR with the participation of major
24 medical centers providing treatment for fetuses with FGR according to the guidelines for
25 obstetrical practice in Japan. Fetuses will be randomized to receive either the conventional
26 management for FGR, according to the guidelines in Japan, or a once-daily treatment with 20
27 mg of tadalafil along with the conventional management, until delivery. Fetal growth velocity
28 from the first day of the protocol-defined treatment to birth (g/day) has been defined as the
29 primary endpoint in this study. To minimize bias in terms of fetal baseline condition and timing
30 of delivery, a fetal indication for delivery is established on the basis of the results from the
31 multicenter survey of VLBW infants in Japan using a network database, in which the 82 level
32 III perinatal centers were registered.² The investigator will evaluate fetal baseline conditions at
33 enrollment and will decide the timing of delivery based on this fetal indication. For other
34 complications such as preterm labor, rupture of the membranes, and hypertensive disorder of
35 pregnancy, the investigator will follow guidelines for obstetric practice in Japan.¹ The
36 investigator will enter the patients' data into the Case Report Form on the website of this
37 clinical trial (the Clinical Trial Data Management System). Infants will be followed up and
38 evaluated for physiological and neurological development until 1.5 years of age.

3. RESEARCH ORGANIZATION AND PARTICIPATING INSTITUTIONS

(1) Corresponding	Mie University	Tomoaki Ikeda (Principal Investigator)
(2) Collaborator	Showa University	Akihiko Sekizawa
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	Kanazawa University	Hiroshi Fujiwara
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16	Kyoto Prefectural University	Jo Kitawaki
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18	Toyama Central Prefectural Hospital	Hiroshi Funamoto
19	Sapporo City General Hospital	Kazuhiko Okuyama
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21	Kagoshima University	Hiroaki Kobayashi
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23	Mie Prefectural General Medical Center	Hirohiko Tanaka
24	Kyoto University	Masaki Mandai
25		
26	Sakakibara Heart Institute	Shinji Katsuragi
27		
28	University of Fukui	Yoshio Yoshida

(3) Safety Evaluation Committee

The Safety Evaluation Committee is independent from research organization, and responsible for the overall safety of this clinical study. To ensure the safety of the protocol-defined treatment, the Safety Evaluation Committee will review adverse events of tadalafil. The Safety Evaluation Committee consists of Dr. Makoto Maeda (Board Certified Member of the Japan Society of Obstetrics and Gynecology) and Dr. Yoshiaki Miyake (Board Certified Member of the Japan Society of Obstetrics and Gynecology).

(4) Protocol Evaluation Committee

The Protocol Evaluation Committee is an organization of the execution of this study. All experimental protocols are evaluated and approved by the Protocol Evaluation Committee.

(5) Data Coordinating Center at the Clinical Research Support Center in Mie University Hospital

This center supports the data management, and statistical analysis and reporting of the study. This consists of Dr. Masakatsu Nishikawa (chairperson), Ms. Yuki Nishimura (data manager), and Dr. Toru Ogura (statistics).

(6) Secretariats

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4. STUDY SUBJECTS AND METHODS

(1) Study Sites and Subjects

1) Study Sites

This is a multicenter randomized controlled phase II trial, in which the Clinical Research Support Center in Mie University Hospital serves as the data center. Since this trial has been designed to prospectively evaluate the efficacy and safety of tadalafil treatment in FGR, fetuses will be randomized to receive either the conventional management for FGR, according to the guidelines in Japan¹ or a once-daily treatment with 20 mg of tadalafil along with the conventional management at the data center. Case registration requires the approval of the Ethics Committee. The following institutions will participate in this clinical trial:

Mie University, Showa University, Osaka University, Nagoya University, Mie Chuo Medical Center, Municipal Yokkaichi hospital, Ise Red Cross Hospital, St. Marianna University, Juntendo University, the Jikei University, Toho University, Yokohama City University Medical Center, Kanagawa Children's Medical Center, Ehime University, Hamamatsu University School of Medicine, Osaka Medical College, Niigata University, Showa University Northern Yokohama Hospital, Showa University Koto Toyosu Hospital, Gifu University, University of the Ryukyus, Shiga University, Shinshu University, Ehime Prefectural Central Hospital, Akita University, Tokyo Metropolitan Bokutoh Hospital, Kyorin University, Tokyo Metropolitan Tama Medical Center, Kuwana East Medical Center, Kanazawa University, Nagasaki Medical Center, University of Toyama, Yamaguchi University, Toyota Memorial Hospital, Kainan Hospital, Dokkyo Medical University, Saga Hospital, Kyoto Prefectural University, Toyama Central Prefectural Hospital, Sapporo City General Hospital, Kagoshima University, Mie Prefectural General Medical Center, Kyoto University, Sakakibara Heart Institute, and University of Fukui.

2) Subjects and Diagnostic Methods

All patients have to meet all inclusion criteria without violating any of the exclusion criteria listed below. All subjects will be followed-up until the end of the study.

Inclusion Criteria

(1) Pregnant women ≥ 20 years.

- (2) EFW less than 1.5 standard deviations of the mean EFW for GA.
- (3) GA between 20 + 0 and 33 + 6 weeks.
- (4) The expected date of confinement is determined using the criteria of the guidelines for obstetrical practice in Japan (2014).
- (5) Singleton pregnancy.
- (6) Signed written informed consent.

Exclusion Criteria

- (1) A result from the antepartum fetal tests, done at enrollment, which indicates that delivery should be attempted.
- (2) A history of allergy to tadalafil.
- (3) Concurrent medications that interact adversely with tadalafil.
- (4) Contraindication of tadalafil treatment due to renal disease.
- (5) Contraindication of tadalafil treatment due to liver disease.
- (6) Contraindication of tadalafil treatment due to uncontrolled arrhythmia, hypertension (BP >170/100 mmHg), and hypotension (BP <80/40 mmHg).
- (7) Fetus with suspected chromosomal disorder and/or multiple congenital anomalies.
- (8) Contraindication of tadalafil treatment due to retinitis pigmentosa, coagulation defect, active gastric and/or intestinal ulcer, and venous obstructive disease.
- (9) The investigator decides to entry inappropriate.

Rationale for Eligibility Criteria

- When diagnosed as FGR, the mean EFW for GA but not the mean birthweight for GA should be used, and the estimated date of confinement using fetal measurements obtained during the early stage of pregnancy should be confirmed according to the guidelines for obstetrical practice in Japan (2014) in Inclusion Criteria Nos. 2 and 4.¹
- The lower age limit (20 weeks gestation) of Inclusion Criterion No. 3 is determined referring to the previous study protocol about the treatment for FGR.²⁴ The upper limit of <34 weeks gestation is based on infant survival rate in the NICU categorized by birth weight and gestational week at birth from the Japanese neonatal research network database (<http://nponrn.umin.jp/index.html> Japanese-only website), in which indicates that treatments are prioritized over elective delivery (Figure 1).

from 60 to 95%. The third group was defined as “Zone 3” where the infant survival rate in the NICU was 95% or higher. All patients in our study will undergo antepartum fetal tests consisting of the evaluation of fetal well-being by ultrasonography, including Doppler imaging of umbilical arterial blood flow, non-stress test, contraction stress test, and biophysical profile scoring depending on GA, to evaluate possible pregnancy termination by the investigator at enrollment (Table 1. Exclusion Criterion No. 1).

Table 1. A fetal indication for delivery in the TADAFER II study.^{1,23,25}

Infant survival rate in the NICU (See Figure 1)	
Zone 1	Decide timing of delivery depending on available therapeutic measures at NICU in each institute.
Zone 2	Consider delivery if at least one of three findings is made, but place give high priority on the determination by the investigators. <ol style="list-style-type: none"> 1. Reversed umbilical artery blood flow during diastole. 2. Score less than 4 on the fetal biophysical profile score. 3. Feat heart rate patterns in the orange or red category for more than 30 minutes.²⁵
Zone 3	Consider delivery if at least one of five findings is made, but place give high priority on the determination by the investigators. <ol style="list-style-type: none"> 1. Reversed or absent umbilical artery blood flow during diastole. 2. Score less than 4 on the fetal biophysical profile score. (Score less than 6 on the fetal biophysical profile score if oligohydramnios is present.) 3. Feat heart rate patterns in the orange or red category for more than 30 minutes.²⁵ 4. Positive contraction stress test. 5. Impaired fetal head circumference growth for more than 2 weeks.

- Patients who have contraindications for tadalafil treatment will be excluded (Exclusion Criteria from No.2 to No.7).
- Regarding exclusion criteria No.9, this study excludes mothers with mental or psychiatric problems, since poor judgment capabilities that are often associated with such conditions may not be compatible with Inclusion Criterion No. 6.

(2) Study Design

This study is a multicenter randomized controlled phase II trial.

(3) Methods

In this multicenter clinical study, each study site will obtain ethics approval of the protocol before its implementation.

Registration

This study protocol defines all the procedures and schedules that the investigator must abide by to complete this clinical study, including patient selection and registration, fetal treatment of FGR, and follow-up (Figure 2).

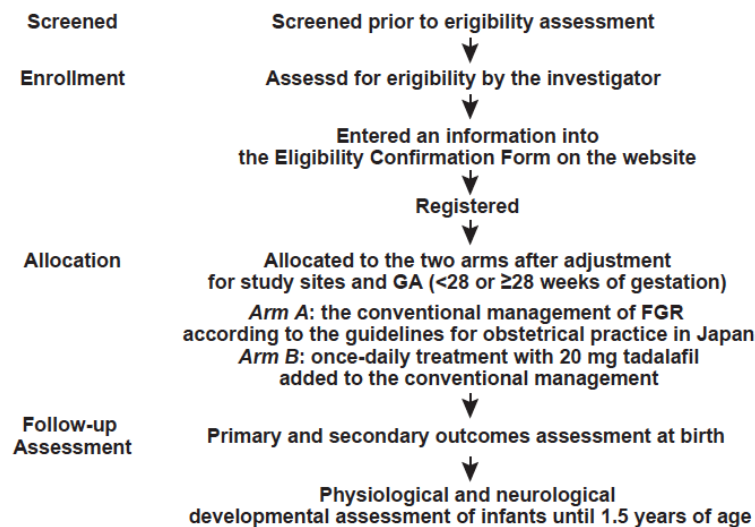


Figure 2. Summary of the study design.

The Clinical Research Support Center in Mie University Hospital will provide data center services including data management and patient registration. Patients that satisfy all inclusion criteria and do not meet any of the exclusion criteria will be eligible for inclusion in the study. Individual study sites will be responsible for guiding potential participants through the informed consent process, including patients who have been referred to them for treatment purposes. The investigator will enter an eligible patient's information into the Eligibility Confirmation Form on the website of this clinical trial (the Clinical Trial Data Management System: Japanese-only website). The data management system will check the contents of the form before registering the patient. For patients who meet all inclusion criteria without violating any of the exclusion criteria listed above, the data management system will register and allocate them to the two arms in an allocation ratio of 1:1, one group receiving the conventional management of FGR according to the guidelines for obstetrical practice in Japan,¹ and the other receiving once-daily treatment with 20 mg tadalafil added to conventional management after adjustment for study

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6 sites and GA (<28 or ≥28 weeks of gestation). The investigators are blinded to the allocation
7 algorithm. Enrolled participants will receive fetal therapy within 7 days of registration. The
8 investigator will enter the patients' data into the Case Report Form on the website of this
9 clinical trial (the Clinical Trial Data Management System).
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12 The corresponding researcher at Mie University will be responsible for the management of this
13 study (patient registration, data management, and coordination with the study-related
14 committees and the Clinical Research Support Center in Mie University Hospital). The
15 corresponding researcher will also be responsible for the research administration, scheduling,
16 documentation, and safety information management. The Safety Evaluation Committee will
17 assume responsibility for the safety of this study. The Clinical Research Support Center in Mie
18 University Hospital will provide technical support from the planning to the completion of this
19 clinical study. Its Data Management Department will manage the study data in cooperation with
20 the corresponding researcher and secretariats, and its Statistics Department will provide
21 statistical support to facilitate the efficacy evaluation. The Protocol Evaluation Committee is an
22 organization of the execution of this study. All experimental protocols are evaluated and
23 approved by the Protocol Evaluation Committee.
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34 **UMIN Clinical Trials Registry UMIN000023778.**

35 36 37 **Fetal Treatment Protocol**

38 The investigator will provide the fetal therapy as described below.

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40 *Arm A:* the conventional management of FGR according to the guidelines for obstetrical
41 practice in Japan.¹ Briefly, the conventional management of FGR consists of evaluation of fetal
42 well-being on ultrasonography, including Doppler imaging of umbilical arterial blood flow,
43 non-stress test, contraction stress test, and biophysical profile scoring depending on GA to
44 evaluate possible pregnancy termination.
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47 *Arm B:* once-daily treatment with 20 mg tadalafil added to the conventional management until
48 delivery.
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51 The investigators are blinded to the allocation algorithm. Enrolled participants will receive fetal
52 therapy within 7 days of registration.
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55 56 **Rationale for Dose Selection**

57 Tadalafil was approved for treatment of erectile dysfunction (ED) in July 2007 in Japan.
58 Nishiuma S *et al.* reported the results from a post marketing surveillance study on tadalafil, with
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6 a primary goal of confirming the safety and effectiveness of tadalafil in Japanese patients with
7 ED in routine clinical practice. 86.7 % of the participants in the surveillance study were
8 prescribed 10mg or 20mg tadalafil daily.²⁶ We referred the results of adverse events in the
9 surveillance study and determined the dose of tadalafil in our retrospective study, in which three
10 pregnant women (27.3%) were prescribed 10 mg tadalafil daily and eight pregnant women
11 (72.7%) were prescribed 20 mg daily.²¹ In our phase I study, more patients who were
12 administered 40 mg tadalafil daily experienced adverse events than those administered 10 mg or
13 20 mg tadalafil daily, but we found that there were no serious maternal adverse events.²² Finally,
14 the minimum required sample size was estimated based on the results of our retrospective study.
15 Taken together, the tadalafil dosage (once-daily treatment with 20 mg) was set in this study.
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24 **Stopping Criteria**

25 The investigator must discontinue the protocol-defined treatment when certain events prevent
26 continuation of the protocol treatment. These events include the following:
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- 29 1. The mother has withdrawn her consent to study participation.
- 30 2. Certain events prevent continuation of the protocol treatment, which include the following:
 - 31 a) A serious adverse drug reaction to tadalafil has developed.
 - 32 b) The investigator's decision to prioritize other management including termination of the
 - 33 pregnancy instead of continuation of the protocol-defined treatment.
 - 34 c) The investigator's decision that it is inappropriate to continue with the protocol treatment.
 - 35 d) The mother's poor compliance or discontinuation of the protocol treatment.

36 Fetuses in whom the fetal therapy was prematurely discontinued as per the Stopping Criteria
37 will receive scheduled examinations and other assessments to the extent possible. If the mother
38 withdraws her consent to study participation, she and her fetus will be removed from the study.
39 If the fetal therapy is prematurely discontinued due to a serious adverse drug reaction to
40 tadalafil, scheduled subsequent examinations and other assessments should be continued to the
41 extent possible and the investigator should provide the patient experiencing an adverse event
42 with the most appropriate therapeutic measures available. If a registered mother or her fetus is
43 found to have been non-conformant to the eligibility criteria, poor compliance and dropping out
44 with the protocol treatment, the mother or fetus will be categorized as noncompliant.
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57 **Criteria for Delivery**

58 In this study, to minimize bias in terms of the timing of delivery, a fetal indication for delivery
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6 is established on the basis of the results from the multicenter survey of VLBW infants in Japan
7 using a network database (Figure 1 and Table 1). After registration, all patients will receive the
8 conventional management of FGR according to the guidelines for obstetrical practice in Japan
9 regardless of the treatment arm.¹ Briefly, the conventional management of FGR consists of the
10 evaluation of fetal well-being on ultrasonography, including Doppler imaging of umbilical
11 arterial blood flow, non-stress test, contraction stress test, and biophysical profile scoring
12 depending on GA, to evaluate possible pregnancy termination. The investigator will evaluate
13 the fetal condition and decide timing of delivery referring to Table 1. For other complications
14 such as preterm labor, rupture of the membranes, and hypertensive disorder of pregnancy, the
15 investigator will follow guidelines for obstetric practice in Japan.¹ The investigator must
16 provide a report that explains the reason for termination of the pregnancy on the website of this
17 clinical trial (the Clinical Trial Data Management System).
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27 **Monitoring Safety during the Fetal Therapy**

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29 The investigator must pay close attention to the safety of not only the fetus but also the mother.
30 As shown in the study schedule, the protocol-defined assessments include evaluation of
31 maternal blood pressure and pulse rate, maternal blood and urine tests (blood
32 fibrinogen and anti-thrombin III levels, liver and renal function tests, serum electrolyte levels,
33 and qualitative urine protein excretion), maternal serum placental growth factor (PIGF) and
34 soluble fms-like tyrosine kinase receptor (sFLT-1) levels. Other assessments include adverse
35 events assessed by medical consultation, and antepartum fetal tests consisting of
36 ultrasonography including Doppler imaging of blood flow (umbilical artery, middle cerebral
37 artery, and uterine artery), non-stress test, contraction stress test, and biophysical profile scoring
38 depending on GA. The investigator will enter patients' safety data into the Case Report Form on
39 the website of this clinical trial (the Clinical Trial Data Management System).
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49 **Safety Evaluation Committee**

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51 The Safety Evaluation Committee is responsible for the overall safety of this clinical study. To
52 ensure the safety of the protocol-defined treatment, the Safety Evaluation Committee will
53 review the adverse events of tadalafil treatment. If a serious adverse event develops, the
54 investigator will provide the Secretariat with the necessary information within 24 hours of its
55 onset, according to the predetermined procedure. The Secretariat then will forward the obtained
56 information without delay to the Safety Evaluation Committee for review. The Safety
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6 Evaluation Committee will notify the investigator of the review results. If the adverse event is
7 definitely or probably related to tadalafil treatment, the Ethics Committee in Mie University
8 Hospital or each institute will consider possible termination of this clinical study. Special
9 attention must be paid to the reporting requirements stipulated in the Ethical Guidelines for
10 Clinical Studies (Ministry of Health, Labor, and Welfare in Japan, 2017). Infants will be
11 followed up and evaluated for physiological and neurological development until 1.5 years of
12 age.
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19 **Note for New Participating Study Sites**

20 This multicenter study is open to new study sites. It is desirable that study sites cooperate with
21 each other. Agreement on this inter-institutional cooperation is a prerequisite for participation in
22 this clinical study. Case registration requires the approval of the Ethics Committee in each
23 institute.
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5. TREATMENT AND COMPENSATION FOR STUDY-RELATED INJURY

Based on our previous studies, we do not expect that serious adverse events will occur frequently in this study.²² However, the investigator may encounter such adverse events as those mentioned in Section 8: Anticipated Adverse Events. The investigator must report adverse drug reactions to the Minister of Health, Labour and Welfare as provided in the Pharmaceuticals and Medical Devices Act. The investigator must also report any serious adverse events without delay to the head of his or her institution, who will in turn forward the information to the Secretariat. The Secretariat will inform the participating study sites of all reported serious adverse events, irrespective of whether expected or unexpected. The Safety Evaluation Committee will review serious adverse event reports and make recommendations to the Principal Investigator, as appropriate. More specifically, the Safety Evaluation Committee will review the information on a serious adverse event that the investigator forwarded as per the predetermined procedure to the Secretariat within 24 hours of its onset. The Safety Evaluation Committee will notify the review results to the investigator. If the adverse event is definitely or probably related to tadalafil treatment, the Ethics Committee in Mie University Hospital or each institute will consider possible termination of this clinical study. Special attention must be paid to the reporting requirements stipulated in the Ethical Guidelines for Clinical Studies (Ministry of Health, Labor, and Welfare in Japan, 2017).

According to the provisions of the Ethical Guidelines for Clinical Studies (Ministry of Health, Labour and Welfare in Japan, 2017), the study site will inform the Ministry of Health, Labour and Welfare of unexpected adverse events whose study causality cannot be denied. The Ministry of Health, Labour and Welfare will announce reported serious adverse drug reactions to the public at regular intervals. The study site must provide the patient experiencing an adverse event with the most appropriate therapeutic measures available. In this clinical study, maternal complications associated with the protocol-defined treatment have been covered by liability insurance. However, because fetal complications associated with the protocol-defined treatment have not been covered by liability insurance, the investigator must describe this issue in the informed consent document. The corresponding researcher at Mie University is responsible for dealing with inquiries from participating study sites. In case of an accident, the corresponding researcher will consult the Ethics Committee in Mie University for guidance. This study will comply with the reporting requirements provided in the Ethical Guidelines for Clinical Studies (Ministry of Health, Labour and Welfare in Japan, 2017).

6. STUDY PERIOD AND TARGET SAMPLE SIZE

(1) Study Period

The planned study period is from date of ethics approval to February 2021. The Patient Registration Period will last until December 2018. The children's outcome will be followed up for 1.5 years after birth. Data collected by the end of the Neonatal Evaluation Period will be subjected to statistical analysis.

Patient Registration Period: date of ethics approval to December 2018.

Children's Outcome Follow-up Period: 1.5 years after the last birth

(2) Target Sample Size

140 fetuses and their mothers

Rationale for the Target Sample Size

Table 2 shows the summary of the distribution of fetal growth velocity from enrollment to birth in our retrospective study.²¹ We estimate that the distribution of fetal growth velocity of this prospective phase II trial will be similar to that of our retrospective study. When the results of our prospective study are analyzed by Wilcoxon Rank Sum Test and group comparisons, with an α of 0.05, two sided, we will have 90% power to detect a difference if we randomize 62 women per group. Allowing for a 10% drop-out rate, the total sample size required is 140 women.

Table 2. The distribution of fetal growth velocity from enrollment to birth in the retrospective study conducted at Mie University Hospital.

Fetal growth velocity (g/day)	<5	≥5 to <10	≥10 to <15	≥15 to <20	≥20 to <25	≥25
The conventional management group (%)	5.3	10.5	21.1	47.3	15.8	0
The tadalafil group (%)	0	8.3	8.3	50.0	16.7	16.7

7. OUTLINE OF THE STUDY PLAN

1. The investigator will register patients with the Clinical Trial Data Management System according to the procedure defined above.
2. The Clinical Trial Data Management System will register and allocate them to the two arms in an allocation ratio of 1:1, one group receiving the conventional management of FGR according to the guidelines for obstetrical practice in Japan,¹ and the other receiving once-daily treatment with 20 mg tadalafil added to conventional management after adjustment for study sites and GA (<28 or ≥28 weeks of gestation).

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6 3. The investigator will conduct the protocol-defined treatment. The Stopping Criteria and the
7 Criteria for Delivery are explained in detail above.

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9 4. Timing and Methods of Evaluation

10 The investigator will evaluate the variables listed below according to the study schedule. The
11 investigator will use the Case Report Form on the website of this clinical trial (the Clinical Trial
12 Data Management System).

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15 5. Variables

16 The following safety and efficacy variables will be statistically analyzed:

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18
19 **Variables**

20
21 **(1) Maternal and Fetal**

22 i) Signs and symptoms

23 Headache, vertigo, flushing, epistaxis, palpitations, anorexia, dyspepsia, diarrhea, nausea,
24 myalgia, arthralgia, dyspnea, and fetal movement counting.

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26
27 ii) Maternal vital signs

28 Blood pressure and pulse rate.

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31 iii) Maternal blood and urine test

32 Complete blood count, blood fibrinogen and anti-thrombin 3 levels, liver and renal function
33 tests, serum electrolyte levels, qualitative urine protein excretion, maternal serum placental
34 growth factor (PIGF), and soluble fms-like tyrosine kinase receptor (sFLT-1) levels.

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37 iv) Fetal ultrasound examination

38 Estimated fetal weight (g), fetal head circumference (cm), deepest amniotic fluid pocket (cm),
39 Doppler imaging of blood flow (umbilical artery, middle cerebral artery, and uterine artery)

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42 v) Obstetrics

43 Onset of obstetrical complications including hypertensive disorder of pregnancy (HDP),
44 treatment for obstetrical complications, indication for delivery, mode of delivery, and placental
45 weight.

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48 vi) Compliance of tadalafil treatment (arm B only).

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50
51 vi) Adverse events

52
53 **(2) Neonatal**

54 i) GA at birth.

55
56 ii) Physical development

57 Body weight, height, head circumference, and percentile of birth weight for GA and sex

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59 iii) Apgar score
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iv) Clinical laboratory testing

Umbilical artery pH and base excess values

v) Admission in the NICU

vi) Neonatal complications

Respiratory distress syndrome (RDS), pulmonary hemorrhage, neonatal pulmonary hypertension, neonatal chronic lung disease, symptomatic patent ductus arteriosus (PDA), late-onset circulatory dysfunction, intraventricular hemorrhage, periventricular leukomalacia, hypoxic-ischemic encephalopathy, sepsis, necrotizing enterocolitis, gastroesophageal reflux, meconium plug syndrome, retinopathy of prematurity (ROP), anemia of prematurity, auditory disorder (abnormal auditory brainstem response results), congenital abnormality, death, and others.

(3) Pediatric

Physiological and neurological developmental assessment until 1.5 years of age, infant complications including cerebral palsy and epilepsy, and death.

Study Endpoints

(1) Primary endpoint

Fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day).

The primary endpoint is fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day), and is calculated using the following formula:

$$\text{Fetal growth velocity (g/day)} = \frac{\text{Birthweight} - \text{EFW at the first day of the treatment [g]}}{\text{Days of the treatment [days]}}$$

Rationale for the primary endpoint

Our primary hypothesis is that tadalafil therapy will increase the likelihood of increased fetal growth velocity in fetuses with FGR. Taking into account the results of our retrospective study demonstrating that tadalafil treatment increased fetal growth velocity (g/day), which was the primary outcome of the retrospective study, and decreased the incidence of RDS, an improvement in fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day) is an important indicator of the therapeutic benefits for fetuses with FGR.²¹

(2) Secondary endpoints

1) Completion rate of the treatment regimen.

Completion rate of the treatment regimen is defined as the percentage of enrolled patients who receive the protocol-defined treatment for more than 7 days.

2) Efficacy endpoints.

i) Estimated fetal weight (g).

Estimated fetal weight (EFW) is calculated using the following formula:²⁷

$$\text{EFW (g)} = 1.07 \times (\text{biparietal diameter: BPD})^3 + 0.3 \times (\text{abdominal circumference: AC})^2 \times (\text{femur length: FL})$$

ii) Fetal growth velocity in the two weeks after the protocol-defined treatment and in the two weeks after one week of the protocol-defined treatment (g/day).

Fetal growth velocity in the two weeks after the protocol-defined treatment (g/day) is calculated using the following formula:

$$\begin{aligned} & \text{Fetal growth velocity in the two weeks after the protocol – defined treatment (g/day)} \\ &= \frac{(\text{EFW two weeks after the treatment} - \text{EFW at the first day of the treatment [g]})}{14 \text{ [days]}} \end{aligned}$$

and fetal growth velocity in the two weeks after one week of the protocol-defined treatment (g/day) is calculated using the following formula:

$$\begin{aligned} & \text{Fetal growth velocity in the two weeks after one week of the treatment (g/day)} \\ &= \frac{(\text{EFW three weeks after the treatment} - \text{EFW one week after the treatment [g]})}{14 \text{ [days]}} \end{aligned}$$

iii) Fetal growth rate in the two weeks after the protocol-defined treatment and from the first day of the protocol-defined treatment to birth (%/day).

Fetal growth rate in the two weeks after the protocol-defined treatment (%/day) is calculated using the following formula:

$$\begin{aligned} & \text{Fetal growth rate in the two weeks after the protocol-defined treatment (%/day)} \\ &= \frac{\frac{\text{EFW two weeks after the treatment} - \text{EFW at the first day of the treatment [g]}}{\text{EFW at the first day of the treatment [g]}} \times 100}{14 \text{ [days]}} \end{aligned}$$

and Fetal growth rate from the first day of the protocol-defined treatment to birth (%/day) is calculated using the following formula:

$$\begin{aligned} & \text{Fetal growth rate from the first day of the protocol-defined treatment to birth (%/day)} \\ &= \frac{\frac{\text{Birthweight} - \text{EFW at the first day of the treatment [g]}}{\text{EFW at the first day of the treatment [g]}} \times 100}{\text{Days of the treatment [days]}} \end{aligned}$$

iv) Fetal head circumference (cm).

The fetal head circumference was measured at the plane of the third ventricle with the thalamus

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6 in the central portion and the cavum septi pellucidi visible in the anterior portion.

7 v) Doppler imaging of umbilical arterial blood flow.

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9 Umbilical arterial blood flow was examined by Doppler ultrasound according to the Society for
10 Maternal-Fetal Medicine (SMFM) Clinical Guideline.²⁸

11
12 vi) Deepest amniotic fluid pocket (cm).

13
14 The deepest amniotic fluid pocket was measured by transabdominal ultrasonography.

15
16 vii) Prolongation of gestational age at birth (days).

17
18 Prolongation of gestational age at birth is defined as days from the first day of the
19 protocol-defined treatment to birth.

20
21 viii) Birth weight (g).

22
23 Birth weight is defined as the weight of the infant at birth.

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25 ix) GA at birth.

26
27 GA at birth is defined as the gestational age at birth.

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29 x) Apgar score.

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31 The Apgar score consists of an evaluation of five factors: heart rate, respiratory effort, muscle
32 tone, responsiveness and color at one minute and five minutes after birth.

33
34 xi) Umbilical artery pH and base excess values.

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36 Umbilical artery pH and base excess is measured at delivery.

37
38 xii) Incidence rate of pre-eclampsia.

39
40 Incidence rate of pre-eclampsia is defined as the percentage of enrolled patients who develop
41 pre-eclampsia after the protocol-defined treatment.

42
43 xiii) Pediatric developmental assessment until 1.5 years of age.

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45 Pediatric developmental assessment includes physiological and neurological developmental
46 assessment, and infant complications including cerebral palsy, epilepsy, and death.

47 3) Safety endpoints

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49 i) Incidence rate of obstetric complications.

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51 Incidence rate of obstetric complications including HDP is defined as the percentage of enrolled
52 patients who develop obstetric complications after the protocol-defined treatment.

53
54 ii) Perinatal mortality.

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56 Perinatal mortality is defined to include stillbirths (occurring after 22 weeks of gestation) and
57 neonatal deaths (occurring up to 7 days after birth).

58
59 iii) Neonatal mortality.

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61 Neonatal mortality is defined as neonatal deaths occurring up to 28 days after birth.

(3) Statistics

Analysis is done on all randomized fetuses who receive the protocol-defined treatment at least once, as the full analysis set. Analysis per protocol set (i.e., removing patients who do not meet the inclusion and exclusion criteria) is done as a secondary analysis population for sensitivity analysis. All outcome measures are presented as summaries of descriptive statistics (mean [SD] or median [minimum and maximum] for continuous measures, and the numbers and proportions for ordinal and dichotomous measures). Descriptive statistics for the primary endpoint, i.e. fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day), are analyzed for each treatment arm by the Wilcoxon Rank Sum Test and group comparisons. All analyses are performed according to a pre-specified statistical analysis plan. The Data Coordinating Center in Mie University Hospital supports the data management, statistical analysis, and reporting of the study.

8. ANTICIPATED ADVERSE EVENTS

Because we have already demonstrated in phase I clinical trial that tadalafil treatment was feasible in pregnant women with FGR,²² tadalafil treatment for FGR can be administered with relative safety and ease. Yet, this therapy may give rise to unexpected adverse events, given the limited clinical experience with this approach and exposure of healthy mothers without pulmonary hypertension to tadalafil. The investigator must fully inform prospective participants of such possibility and administer the fetal therapy with careful attention and monitoring. Adverse reactions to tadalafil divided into the four groups by the frequency (Very common [$\geq 1/10$], common [$\geq 1/100$ to $< 1/10$], uncommon [$\geq 1/1,000$ to $< 1/100$], and not known [cannot be estimated from the available data]) described in the product information of tadalafil (ADCIRCA® 20 mg tablets) are shown below:²⁹

- Very common ($\geq 1/10$)
Headache, flushing, nasopharyngitis, nausea, dyspepsia, myalgia, neck pain, and pain in extremity.
- Common ($\geq 1/100$ to $< 1/10$)
Hypersensitivity reactions*, syncope, migraine*, blurred vision, palpitations* ***, hypotension, epistaxis, vomiting, gastroesophageal reflux, rash, increased uterine bleeding**, facial oedema, and chest pain***.
- Uncommon ($\geq 1/1,000$ to $< 1/100$)

Seizures*, transient amnesia*, tinnitus, Sudden cardiac death****, Tachycardia****, hypertension, urticaria*, hyperhidrosis*, haematuria, priapism*, penile haemorrhage, and haemospermia

- Not known (cannot be estimated from the available data)
Angioedema, stroke***, non-arteritic anterior ischemic optic neuropathy, retinal vascular occlusion, visual, field defect, sudden hearing loss, unstable angina pectoris, ventricular arrhythmia, myocardial infarction***, Stevens-Johnson Syndrome, exfoliative dermatitis, and prolonged erections.

* The adverse reactions have been included in the table as a result of postmarketing or clinical study data from the use of tadalafil in the treatment of erectile dysfunction; and in addition, the frequency estimates are based on only 1 or 2 patients experiencing the adverse reaction in the pivotal placebo controlled study of ADCIRCA®.

** Clinical non-Medical Dictionary for Regulatory Activities (MedDRA) term to include reports of abnormal/excessive menstrual bleeding, conditions such as menorrhagia, metrorrhagia, menometrorrhagia, or vaginal hemorrhage.

***Most of the patients in whom these events have been reported had pre-existing cardiovascular risk factors.

9. POTENTIAL BENEFITS AND RISKS

(1) Benefits

Potential benefits of this study include cure or improvement in FGR.

(2) Risks

Maternal exposure to tadalafil is inevitable in patients allocated tadalafil treatment arm. Therefore, precautions must ensure the safety of both the mother and the fetus. Specific descriptions of such risks have been described in Section 8: Anticipated Adverse Events. To control for such risks, this study has stipulated an array of tests, such as hematology, serum chemistry, medical consultation, and antepartum fetal tests consisting of ultrasonography including Doppler imaging of blood flow (umbilical artery, middle cerebral artery, and uterine artery), non-stress test, contraction stress test, and biophysical profile scoring depending on GA. In the event of an adverse drug reaction, the investigator will immediately take appropriate measures, possibly including early withdrawal from the study. The investigator must prioritize maternal safety over fetal therapy. If the mother develops an adverse drug reaction, it will be treated under liability insurance and / or the national health insurance scheme.

10. BURDEN OF COST

This research was supported by by the Japan Agency for Medical Research and Development (AMED). This fund will be paid for items related to research (purchasing cost for tadalafil, data management, storage, analysis, etc.) other than medical examination. Medical examination expenses are covered by the national health insurance scheme.

11. INTELLECTUAL PROPERTY RIGHTS

Any intellectual property rights that may arise from this clinical study shall be exclusively owned by the TADAFER study group. The corresponding researcher and the joint researchers report no conflicts of interest related to this clinical study or to their organizations.

12. ETHICS

This clinical study focuses on prenatal treatment, and its protocol has been developed according to the Ethical Guidelines for Clinical Studies (Ministry of Health, Labour and Welfare in Japan, 2017). Before the start of this clinical study, the corresponding researcher will explain its objectives and outline them fully to the participating site investigators. We believe that application of the guideline requirements to the mother who consents to participate in this study will ensure that her fetus is also protected by the ethical principles of the guidelines. As per the Ethical Guidelines for Clinical Studies, participation in this study will be preceded by the informed consent process. Considering the difficulty in obtaining assent, even implicitly, from the fetus, we believe that the parental permission for the fetus to participate.

13. ADDITIONAL NOTES RELATED TO THE ACT ON THE PROTECTION OF PERSONAL INFORMATION RELATED BY INDEPENDENT ADMINISTRATIVE INSTITUTIONS

1. Data Collection

Study data will be de-identified before being stored in electronic format. De-identified or anonymous data will be analyzed at Mie University. Joint researchers will examine and discuss the analyzed results.

2. Data Management

The results of analyses of the collected test data will be securely stored at the Secretariat located in Mie University.

3. Storage of Electronic Media

The results of analyses will be filed in electronic media, which will be kept securely in a locked room of Mie University. The Secretariat staff member, Dr. Takashi Umekawa, assumes the responsibility for data storage. In addition to the corresponding researcher, appointed members of the Secretariat staff will be granted access to the study data.

4. Method and Timing of Data De-identification

Registration numbers will be used to de-identify the study data at individual study sites. Each study site must ensure that the data they transfer to the Secretariat contains no explicit personal identifiers.

5. Notification of Analytical Results

Parents who participate in this study will not be informed of the results of this study.

14. REFERENCE

- 1 Minakami H, Maeda T, Fujii T, Hamada H, Iitsuka Y, Itakura A *et al.*
Guidelines for obstetrical practice in Japan: Japan Society of Obstetrics and Gynecology
(JSOG) and Japan Association of Obstetricians and Gynecologists (JAOG) 2014 edition. *J*
Obstet Gynaecol Res 2014; **40**: 1469–1499.
- 2 Kusuda S, Fujimura M, Sakuma I, Aotani H, Kabe K, Itani Y *et al.*
Morbidity and mortality of infants with very low birth weight in Japan: center variation.
Pediatrics 2006; **118**: e1130–e1138.
- 3 American College of Obstetricians and Gynecologists. ACOG Practice
bulletin no. 134: fetal growth restriction. *Obstet Gynecol* 2013; **121**: 1122–1133.
- 4 Hui L, Challis D. Diagnosis and management of fetal growth restriction: the
role of fetal therapy. *Best Pract Res Clin Obstet Gynaecol* 2008; **22**: 139–158.
- 5 Coppage KH, Sun X, Baker RS, Clark KE. Expression of phosphodiesterase
5 in maternal and fetal sheep. *Am J Obstet Gynecol* 2005; **193**: 1005–1010.
- 6 Rotella DP. Phosphodiesterase 5 inhibitors: current status and potential
applications. *Nat Rev Drug Discov* 2002; **1**: 674–682.
- 7 Wareing M, Myers JE, O’Hara M, Kenny LC, Warren AY, Taggart MJ *et al.*
Effects of a phosphodiesterase-5 (PDE5) inhibitor on endothelium-dependent relaxation of
myometrial small arteries. *Am J Obstet Gynecol* 2004; **190**: 1283–1290.
- 8 Wareing M, Myers JE, O’Hara M, Baker PN. Sildenafil citrate (Viagra)
enhances vasodilatation in fetal growth restriction. *J Clin Endocrinol Metab* 2005; **90**: 2550–
2555.
- 9 Herraiz S, Pellicer B, Serra V, Cauli O, Cortijo J, Felipe V *et al.* Sildenafil
citrate improves perinatal outcome in fetuses from pre-eclamptic rats. *BJOG Int J Obstet*
Gynaecol 2012; **119**: 1394–1402.
- 10 Ramesar SV, Mackraj I, Gathiram P, Moodley J. Sildenafil citrate improves
fetal outcomes in pregnant, L-NAME treated, Sprague-Dawley rats. *Eur J Obstet Gynecol*
Reprod Biol 2010; **149**: 22–26.
- 11 Baijnath S, Soobryan N, Mackraj I, Gathiram P, Moodley J. The
optimization of a chronic nitric oxide synthase (NOS) inhibition model of pre-eclampsia by
evaluating physiological changes. *Eur J Obstet Gynecol Reprod Biol* 2014; **182**: 71–75.
- 12 Nassar AH, Masrouha KZ, Itani H, Nader KA, Usta IM. Effects of sildenafil
in N ω -nitro-L-arginine methyl ester-induced intrauterine growth restriction in a rat model. *Am J*

1
2
3
4
5
6 *Perinatol* 2012; **29**: 429–434.

7 13 Cross JC, Hemberger M, Lu Y, Nozaki T, Whiteley K, Masutani M *et al.*
8 Trophoblast functions, angiogenesis and remodeling of the maternal vasculature in the placenta.
9 *Mol Cell Endocrinol* 2002; **187**: 207–212.

10 14 Watson ED, Cross JC. Development of structures and transport functions in
11 the mouse placenta. *Physiol Bethesda Md* 2005; **20**: 180–193.

12 15 von Dadelszen P, Dwinnell S, Magee LA, Carleton BC, Gruslin A, Lee B *et*
13 *al.* Sildenafil citrate therapy for severe early-onset intrauterine growth restriction. *BJOG Int J*
14 *Obstet Gynaecol* 2011; **118**: 624–628.

15 16 Sharp A, Comforth C, Jackson R, Turner M, Kenny L, Baker P *et al.*
16 OC01.05: STRIDER UK: a randomised controlled trial of sildenafil therapy in dismal prognosis
17 early-onset intrauterine growth restriction. *Ultrasound Obstet Gynecol* 2017; **50**: 3–3.

18 17 Sahni S, Palkar AV, Rochelson BL, Kępa W, Talwar A. Pregnancy and
19 pulmonary arterial hypertension: A clinical conundrum. *Pregnancy Hypertens Int J Womens*
20 *Cardiovasc Health* 2015; **5**: 157–164.

21 18 Wilkins MR, Wharton J, Grimminger F, Ghofrani HA. Phosphodiesterase
22 inhibitors for the treatment of pulmonary hypertension. *Eur Respir J* 2008; **32**: 198–209.

23 19 Fogue ST, Patterson BE, Bedding AW, Payne CD, Phillips DL, Wrishko
24 RE *et al.* Tadalafil pharmacokinetics in healthy subjects. *Br J Clin Pharmacol* 2006; **61**: 280–
25 288.

26 20 Yoshikawa K, Umekawa T, Maki S, Kubo M, Nii M, Tanaka K *et al.*
27 Tadalafil Improves L-NG-Nitroarginine Methyl Ester-Induced Preeclampsia With Fetal Growth
28 Restriction-Like Symptoms in Pregnant Mice. *Am J Hypertens In press.*

29 21 Kubo M, Umekawa T, Maekawa Y, Tanaka H, Nii M, Murabayashi N *et al.*
30 Retrospective study of tadalafil for fetal growth restriction: Impact on maternal and perinatal
31 outcomes. *J Obstet Gynaecol Res* 2017; **43**: 291–297.

32 22 Kubo M, Tanaka H, Maki S, Nii M, Murabayashi N, Osato K *et al.* Safety
33 and dose-finding trial of tadalafil administered for fetal growth restriction: A phase-1 clinical
34 study. *J Obstet Gynaecol Res* 2017; **43**: 1159–1168.

35 23 Cunningham F, Leveno K, Bloom S, Spong CY, Dashe J. *Williams*
36 *Obstetrics, 24e.* McGraw-Hill, 2014.

37 24 Ganzevoort W, Alfirovic Z, von Dadelszen P, Kenny L, Papageorgiou A,
38 van Wassenaer-Leemhuis A *et al.* STRIDER: Sildenafil Therapy In Dismal prognosis
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6 Early-onset intrauterine growth Restriction—a protocol for a systematic review with individual
7 participant data and aggregate data meta-analysis and trial sequential analysis. *Syst Rev* 2014; **3**:
8 23.
- 9
10
11 25 Parer JT, Ikeda T. A framework for standardized management of intrapartum
12 fetal heart rate patterns. *Am J Obstet Gynecol* 2007; **197**: 26–e1.
- 13
14 26 Shinichi Nishiuma, Kimiko Arakawa, Masanori Taketsuna, Nobuyuki
15 Kobayashi. Safety and effectiveness of tadalafil in patients with erectile dysfunction based on
16 post marketing surveillance study. *Jpn J Impot Res* 2012; **27**: 15–26.
- 17
18
19 27 Shinozuka N. Fetal biometry and fetal weight estimation: JSUM
20 standardization. *Ultrasound Rev Obstet Gynecol* 2002; **2**: 156–161.
- 21
22 28 Berkley E, Chauhan SP, Abuhamad A, Committee S for M-FMP. Doppler
23 assessment of the fetus with intrauterine growth restriction. *Am J Obstet Gynecol* 2012; **206**:
24 300–308.
- 25
26
27 29 ADCIRCA, INN - Tadalafil - WC500032789.pdf.
28 [http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/huma](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/001021/WC500032789.pdf)
29 [n/001021/WC500032789.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/001021/WC500032789.pdf) (accessed 19 Nov2017).
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CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4-6
	2b	Specific objectives or hypotheses	6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6-8
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	N/A
Participants	4a	Eligibility criteria for participants	6-7
	4b	Settings and locations where the data were collected	8 and 14
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6-8 and 11
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	8-11
	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample size	7a	How sample size was determined	13
	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	8
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	8
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	8
	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	8
Implementation	11	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	N/A
Blinding			

1		11	If relevant, description of the similarity of interventions	8
2		b		
3	Statistical	12	Statistical methods used to compare groups for primary and	13
4	methods	a	secondary outcomes	
5		12	Methods for additional analyses, such as subgroup analyses and	N/A
6		b	adjusted analyses	
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8	Results			
9	Participant flow	13	For each group, the numbers of participants who were randomly	N/A
10	(a diagram is	a	assigned, received intended treatment, and were analysed for	
11	strongly		the primary outcome	
12	recommended)	13	For each group, losses and exclusions after randomisation,	N/A
13		b	together with reasons	
14	Recruitment	14	Dates defining the periods of recruitment and follow-up	N/A
15		a		
16		14	Why the trial ended or was stopped	N/A
17		b		
18	Baseline data	15	A table showing baseline demographic and clinical	N/A
19			characteristics for each group	
20	Numbers	16	For each group, number of participants (denominator) included	N/A
21	analysed		in each analysis and whether the analysis was by original	
22			assigned groups	
23	Outcomes and	17	For each primary and secondary outcome, results for each	N/A
24	estimation	a	group, and the estimated effect size and its precision (such as	
25			95% confidence interval)	
26		17	For binary outcomes, presentation of both absolute and relative	N/A
27		b	effect sizes is recommended	
28	Ancillary	18	Results of any other analyses performed, including subgroup	N/A
29	analyses		analyses and adjusted analyses, distinguishing pre-specified	
30			from exploratory	
31	Harms	19	All important harms or unintended effects in each group (for specific	N/A
32			guidance see CONSORT for harms)	
33				
34	Discussion			
35	Limitations	20	Trial limitations, addressing sources of potential bias,	14-15
36			imprecision, and, if relevant, multiplicity of analyses	
37	Generalisability	21	Generalisability (external validity, applicability) of the trial	14-15
38			findings	
39	Interpretation	22	Interpretation consistent with results, balancing benefits and	14-15
40			harms, and considering other relevant evidence	
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42	Other information			
43	Registration	23	Registration number and name of trial registry	13
44	Protocol	24	Where the full trial protocol can be accessed, if available	N/A
45	Funding	25	Sources of funding and other support (such as supply of drugs),	15
46			role of funders	

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

BMJ Open

TADAFER II: Tadalafil treatment for fetal growth restriction - a study protocol for a multicenter randomized controlled phase II trial.

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Manuscript ID	bmjopen-2017-020948.R2
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Primary Subject Heading:	Obstetrics and gynaecology

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Secondary Subject Heading:	Obstetrics and gynaecology, Research methods
Keywords:	Fetal growth restriction, Phosphodiesterase 5 inhibitor, Tadalafil, Phase II trial, Study protocol

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1 **Title: TADAFER II: Tadalafil treatment for fetal growth restriction - a study protocol for**
2 **a multicenter randomized controlled phase II trial.**

3
4 Running head: Tadalafil for fetal growth restriction

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29 **Disclosure**

30 The authors declare no conflict of interest.

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32 **Word count:** 4495 words.

1 **Title: TADAFER II: Tadalafil treatment for fetal growth restriction - a study protocol for**
2 **a multicenter randomized controlled phase II trial.**

3
4 **ABSTRACT**

5 **Introduction:** There is no proven therapy to reverse or ameliorate fetal growth restriction
6 (FGR). Sildenafil, a selective phosphodiesterase 5 (PDE5) inhibitor, has been reported to
7 potentially have a therapeutic role in FGR, but this has not been established. Tadalafil is also a
8 selective PDE5 inhibitor. We have demonstrated efficacy for tadalafil on fetal growth in FGR
9 and the short-term outcomes and feasibility of tadalafil in FGR. Based on the hypothesis that
10 tadalafil will safely increase the likelihood of increased fetal growth in FGR, we have designed
11 this phase II study to prospectively evaluate the efficacy and safety of tadalafil in FGR.

12 **Methods and analysis:** This study is a multicenter randomized controlled phase II trial. A total
13 of 140 fetuses with FGR will be enrolled from medical centers in Japan. Fetuses will be
14 randomized to receive either the conventional management for FGR, according to the guidelines,
15 or a once daily treatment with 20 mg of tadalafil along with the conventional management, until
16 delivery. The primary endpoint is fetal growth velocity from the first day of the protocol-defined
17 treatment to birth (g/day), and is calculated using the following formula:

$$\text{Fetal growth velocity (g/day)} = \frac{\text{Birthweight} - \text{Estimated fetal weight at the first day of the treatment [g]}}{\text{Days of the treatment [days]}}$$

18 To minimize bias in terms of fetal baseline conditions and timing of delivery, a fetal indication
19 for delivery is established in this study. The investigator will evaluate fetal baseline conditions
20 at enrollment and will decide the timing of delivery based on this fetal indication. Infants will be
21 followed up for development until 1.5 years of age.

22 **Ethics and dissemination:** This study was approved by the Institutional Review Board of Mie
23 University Hospital and each participating institution. Our findings will be widely disseminated
24 through peer-reviewed publications.

25 **Trial registration:** UMIN000023778.

1 **Strengths and limitations of this study**

- 2 • This is a multicenter randomized controlled phase II trial to prospectively evaluate the
3 efficacy and safety of tadalafil treatment in fetuses with fetal growth restriction (FGR), for
4 which there is no proven therapy.
- 5 • This trial will include the participation of major medical centers providing treatment for
6 fetuses with FGR according to the guidelines for obstetrical practice in Japan.
- 7 • To minimize bias in terms of fetal baseline conditions and timing of delivery, a fetal
8 indication for delivery is established in this study on the basis of the results from a
9 multicenter survey in Japan.
- 10 • The possible limitation is related to open-label trial features, in which enrolled participants
11 receive either the conventional management for FGR according to the guidelines for
12 obstetrical practice in Japan, or a once daily treatment with 20 mg of tadalafil added to the
13 conventional management.
- 14 • It is possible that SGA was included among the cases of FGR without Doppler
15 abnormalities in this study.

1 INTRODUCTION

2 Neonatal intensive care has improved over the past few decades, and morbidity among
3 infants, including those who are premature, continues to decline. Premature infants with
4 intrauterine growth restriction, however, still have high mortality and morbidity. The multicenter
5 survey[1] of very low birth weight (VLBW) infants in Japan conducted by Kusuda and Ikeda *et*
6 *al.* revealed that mortality in neonatal intensive care units (NICU), of small gestational age
7 (SGA) infants born before 30 weeks gestation, was significantly higher than that of appropriate
8 for gestational age (AGA) infants (unpublished data). To prevent fetal growth restriction (FGR),
9 nutritional and dietary supplementation, bed rest, and aspirin therapy have been investigated,
10 but there is insufficient evidence for the routine indication of any of these treatments.[2] There
11 is also no proven therapy to reverse or ameliorate established FGR.[3]

12 Increases in uteroplacental blood flow during pregnancy via angiogenesis and
13 vasodilation contribute to adequate fetal growth. Vasodilation in the uteroplacental unit is
14 considered to be due to the production and local release of nitric oxide (NO), which stimulates
15 cyclic guanosine monophosphate (cGMP) production.[4] cGMP is inactivated mainly by
16 phosphodiesterases (PDE), and the predominant PDE isoform present in the vascular smooth
17 muscle is PDE5. Because inhibitors of PDE5, which is a cGMP-specific PDE, exert their
18 pharmacological action by dilating arteries and increasing blood flow, as proven in erectile
19 dysfunction and pulmonary hypertension, recent studies have suggested a potential therapeutic
20 role for PDE5 inhibitors in treating FGR.[5] Sildenafil, a selective PDE5 inhibitor, has been
21 shown to improve endothelial function in myometrial small arteries removed from women with
22 pre-eclampsia and FGR.[6, 7] However, although sildenafil has been reported to affect maternal
23 hypertension, it has not been shown to affect FGR in studies in FGR model rats induced by
24 L-NG-nitroarginine methyl ester (L-NAME) treatment. In these studies, FGR was not affected
25 by sildenafil except in one report, by Baijnath *et al.*[8-11] Baijnath *et al.* demonstrated that
26 L-NAME-induced FGR was improved by sildenafil treatment from 4 days postcoitum (d.p.c.) to
27 8 d.p.c. but not from 8 d.p.c. to 14 d.p.c.[10] Chorioallantoic attachment occurs at 8 d.p.c., and
28 the mature circulatory pattern of maternal blood through the placenta is established by 10 d.p.c.
29 in mouse placenta.[12, 13] In considering the development of fetoplacental circulation in
30 rodents, the effect of sildenafil on fetal growth associated with placental blood flow via an
31 NO-dependent pathway was not manifested. In a clinical study, it was reported that sildenafil
32 was associated with increased fetal abdominal circumference (AC) growth velocity in severe

1 early-onset FGR, but the authors did not report on fetal growth velocity and birth weight.[14]
2 Recently, the STRIDER UK group has found no evidence of a beneficial effect of sildenafil on
3 survival or short-term neonatal outcomes.[15]

4 Tadalafil is also a selective PDE5 inhibitor and has a longer half-life and a more rapid
5 onset of action than sildenafil.[5] Tadalafil has been used to treat pulmonary hypertension in
6 pregnant women and the Food and Drug Administration in the United States has rated tadalafil
7 as pregnancy category B.[16] Ladouceur *et al.* reported pregnancy outcomes in patients with
8 pulmonary arterial hypertension associated with congenital heart disease treated with tadalafil.
9 They did not describe adverse effects associated with tadalafil.[17] Doimon *et al.* also reported
10 no side effects of tadalafil on mothers or offsprings in cases with pulmonary arterial
11 hypertension treated with tadalafil.[18] When taking sildenafil with a high-fat meal, the time to
12 maximum plasma concentration increases and the peak plasma concentration falls.[19] In
13 contrast, Fogue *et al.* reported that food intake had a negligible effect on the bioavailability of
14 tadalafil, and also reported that there was no clinically meaningful effect of gender on tadalafil
15 pharmacokinetics.[20] Our animal experiments demonstrated that tadalafil treatment dilates the
16 maternal blood sinuses in the placenta, which leads to increased placental growth factor (PlGF)
17 production, and contributes to facilitating fetal growth.[21] Because tadalafil treatment was
18 started after blood spaces in the placenta were narrowed by L-NAME treatment and elevated
19 urinary excretion of cGMP in these animal experiments, we can safely presume that tadalafil
20 treatment contributes to facilitating fetal growth in the context of the mechanisms associated
21 with NO signaling. In addition, we retrospectively analyzed 11 Japanese singleton pregnant
22 women with FGR who received tadalafil along with conventional management for FGR at Mie
23 University Hospital from July 2015 to February 2016 (tadalafil group).[22] These women were
24 matched for maternal age, parity, gestational age (GA), and estimated fetal weight at enrollment
25 with 14 singleton pregnant women who had received only the conventional management for
26 FGR in 2014 (conventional management group). The conventional management for FGR was
27 performed according to the guidelines for obstetric practice in Japan.[23] This retrospective
28 study showed that both fetal growth velocity from enrollment to birth and birth weight were
29 significantly higher in the tadalafil group than in the conventional management group. Moreover,
30 the prevalence of respiratory distress syndrome (RDS) was significantly lower in the tadalafil
31 group than in the conventional management group. After the retrospective study, we conducted
32 a phase I clinical trial to ensure the safety of tadalafil treatment for FGR.[24] There were no

1 serious maternal adverse events for daily tadalafil doses of 10 mg, 20 mg, and 40 mg. More
2 patients who were administered 40 mg tadalafil daily experienced mild adverse events than
3 those administered 10 mg or 20 mg tadalafil daily. In regards to fetal adverse events,
4 intrauterine fetal death occurred in one case. In this case, the pregnant woman was prescribed 40
5 mg tadalafil daily and fetal growth had been progressing at a rate of 22 g/day. At 36 weeks
6 gestation, fetal movement suddenly ceased and a diagnosis of intrauterine fetal death was made.
7 Thereafter, the fetus was delivered vaginally, and velamentous insertion of the umbilical cord
8 was identified. Immediately, the safety evaluation committee investigated the incident's
9 relationship to tadalafil. This committee analyzed the case and concluded that the intrauterine
10 fetal death was due to velamentous insertion of the umbilical cord.[25] We concluded that
11 tadalafil treatment was feasible in pregnant women with FGR.[24]

12 Based on the above, we have hypothesized that tadalafil therapy will safely increase the
13 likelihood of increased fetal growth in fetuses with FGR and have designed this multicenter
14 randomized controlled phase II study to establish evidence for fetal therapy with tadalafil. This
15 study, funded by the Japan Agency for Medical Research and Development (AMED), will
16 prospectively evaluate the safety and efficacy of tadalafil in FGR with the participation of major
17 medical centers providing treatment for fetuses with FGR according to the guidelines for
18 obstetrical practice in Japan.

19 **METHODS**

20 **Study design**

21 This study is a multicenter randomized controlled phase II trial.

22 **Study period**

23 The planned study period is from the date of ethics approval to February 2021. The
24 Patient Registration Period will last until December 2018. The children's outcome will be
25 followed up for 1.5 years after birth. Data collected by the end of the Neonatal Evaluation
26 Period will be subjected to statistical analysis.

27 Patient Registration Period: date of ethics approval to December 2018.

28 Children's Outcome Follow-up Period: 1.5 years after the last birth.

29 **Patient selection**

30 Inclusion criteria are as follows: (1) Pregnant women \geq 20 years; (2) Estimated fetal
31 weight (EFW) less than 1.5 standard deviations of the mean EFW for GA; (3) GA between 20 +

0 and 33 + 6 weeks; (4) The expected date of confinement is determined using the criteria of the guidelines for obstetrical practice in Japan (2014); (5) Singleton pregnancy; and (6) Signed written informed consent.

Exclusion criteria are as follows; (1) A result from the antepartum fetal tests, done at enrollment, which indicates that delivery should be attempted*; (2) A history of allergy to tadalafil; (3) Concurrent medications that interact adversely with tadalafil; (4) Contraindication of tadalafil treatment due to renal disease; (5) Contraindication of tadalafil treatment due to liver disease; (6) Contraindication of tadalafil treatment due to uncontrolled arrhythmia, hypertension (BP >170/100 mmHg), and hypotension (BP <80/40 mmHg); (7) Fetus with suspected chromosomal disorder and/or multiple congenital anomalies; (8) Contraindication of tadalafil treatment due to retinitis pigmentosa, coagulation defect, active gastric and/or intestinal ulcer, or venous obstructive disease; and (9) The investigator decides that entry is inappropriate**.

* To minimize bias in terms of fetal baseline condition at enrollment, a fetal indication for delivery is established on the basis of the results from the multicenter survey of VLBW infants in Japan using a network database, in which the 82 level III perinatal centers were registered. The survey data included infant survival rate in the NICU, categorized by birth weight and gestational week at birth (Figure 1).[1] The infant survival rate data acquired from the survey were preprocessed with the moving average method and divided into three groups. The first group was defined as “Zone 1” where the infant survival rate in the NICU was less than 60%. The second group was defined as “Zone 2” where the infant survival rate in the NICU ranged from 60 to 95%. The third group was defined as “Zone 3” where the infant survival rate in the NICU was 95% or higher. All patients in our study will undergo antepartum fetal tests consisting of the evaluation of fetal well-being by ultrasonography, including Doppler imaging of umbilical arterial blood flow, non-stress test, contraction stress test, and biophysical profile scoring depending on GA, to evaluate possible pregnancy termination by the investigator at enrollment (Table 1). [23, 25, 26]

Table 1. A fetal indication for delivery in the TADAFER II study. [23, 25, 26]

Infant survival rate in the NICU (See Figure 1)	
Zone 1	Decide timing of delivery depending on available therapeutic measures at the NICU in each institute.
Zone 2	Consider delivery if at least one of three findings is made, but place give high priority on the determination by the investigators. <ol style="list-style-type: none"> 1. Reversed umbilical artery blood flow during diastole. 2. Score less than 4 on the fetal biophysical profile score. 3. Feat heart rate patterns in the orange or red category for more than 30 minutes. [26]
Zone 3	Consider delivery if at least one of five findings is made, but place give high priority on the determination by the investigators. <ol style="list-style-type: none"> 1. Reversed or absent umbilical artery blood flow during diastole.

	2.	Score less than 4 on the fetal biophysical profile score. (Score less than 6 on the fetal biophysical profile score if oligohydramnios is present.)
	3.	Feat heart rate patterns in the orange or red category for more than 30 minutes. [26]
	4.	Positive contraction stress test.
	5.	Impaired fetal head circumference growth for more than 2 weeks.

**Regarding exclusion criteria No.9 “The investigator decides that entry is inappropriate”, this study excludes mothers with mental or psychiatric problems, since poor judgment capabilities that are often associated with such conditions may not be compatible with Inclusion Criterion No. 6 “Signed written informed consent”.

Registration

The study protocol defines all of the procedures and schedules that the investigator must abide by to complete this clinical study, including patient selection and registration, fetal treatment of FGR, and follow-up (Figure 2). Patients that satisfy all inclusion criteria and do not meet any of the exclusion criteria will be eligible for inclusion in the study. Individual study sites will be responsible for guiding potential participants through the informed consent process, including patients who have been referred to them for treatment purposes. The investigator will enter an eligible patient’s information into the Eligibility Confirmation Form on the website of this clinical trial (the Clinical Trial Data Management System: Japanese-only website). The data management system will check the contents of the form before registering the patient. For patients who meet all inclusion criteria without violating any of the exclusion criteria listed above, the data management system will register and allocate them to the two arms in an allocation ratio of 1:1, one group receiving the conventional management of FGR according to the guidelines for obstetrical practice in Japan,[23] and the other receiving once-daily treatment with 20 mg tadalafil added to conventional management after adjustment for study sites and GA (<28 or ≥28 weeks of gestation). The investigators are blinded to the allocation algorithm. Enrolled participants will receive fetal therapy within 7 days of registration. The investigator will enter the patients’ data into the Case Report Form on the website of this clinical trial (the Clinical Trial Data Management System).

Fetal Treatment Protocol

The investigator will provide the fetal therapy as described below.

Arm A: the conventional management of FGR according to the guidelines for obstetrical practice in Japan.[23] Briefly, the conventional management of FGR consists of evaluation of fetal well-being on ultrasonography, including Doppler imaging of umbilical arterial blood flow, non-stress test, contraction stress test, and biophysical profile scoring depending on GA to evaluate possible pregnancy termination.

1 *Arm B*: once-daily treatment with 20 mg tadalafil added to the conventional management until
2 delivery.

3 The investigators are blinded to the allocation algorithm. Enrolled participants will receive fetal
4 therapy within 7 days of registration.

5 6 **Endpoints**

7 **(1) Primary endpoint**

8 Fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day).

9 The primary endpoint is fetal growth velocity from the first day of the protocol-defined
10 treatment to birth (g/day), and is calculated using the following formula:

$$11 \quad \text{Fetal growth velocity (g/day)} \\ 12 \quad = \frac{\text{Birthweight} - \text{EFW at the first day of the treatment [g]}}{\text{Days of the treatment [days]}}$$

11 **Rationale for the primary endpoint**

12 Our primary hypothesis is that tadalafil therapy will increase the likelihood of increased
13 fetal growth velocity in fetuses with FGR. Taking into account the results of our retrospective
14 study demonstrating that tadalafil treatment increased fetal growth velocity (g/day), which was
15 the primary outcome of the retrospective study, and decreased the incidence of RDS, an
16 improvement in fetal growth velocity from the first day of the protocol-defined treatment to
17 birth (g/day) is an important indicator of the therapeutic benefits for fetuses with FGR.[22] The
18 cases of fetal death were excluded in analysis of primary endpoint.

19 **(2) Secondary endpoints**

20 1) Completion rate of the treatment regimen.

21 Completion rate of the treatment regimen is defined as the percentage of enrolled patients who
22 receive the protocol-defined treatment for more than 7 days.

23 2) Efficacy endpoints.

24 i) Estimated fetal weight (g).

25 Estimated fetal weight (EFW) is calculated using the following formula:[27]

$$26 \quad \text{EFW (g)} = 1.07 \times (\text{biparietal diameter: BPD})^3 + 0.3 \\ 27 \quad \times (\text{abdominal circumference: AC})^2 \times (\text{femur length: FL})$$

28 ii) Fetal growth velocity in the two weeks after the protocol-defined treatment and in the two
29 weeks after one week of the protocol-defined treatment (g/day).

Fetal growth velocity in the two weeks after the protocol-defined treatment (g/day) is calculated
using the following formula:

Fetal growth velocity in the two weeks after the protocol – defined treatment (g/day)

$$= \frac{(\text{EFW two weeks after the treatment} - \text{EFW at the first day of the treatment [g]})}{14 \text{ [days]}}$$

and fetal growth velocity in the two weeks after one week of the protocol-defined treatment (g/day) is calculated using the following formula:

$$\text{Fetal growth velocity in the two weeks after one week of the treatment (g/day)} \\ = \frac{(\text{EFW three weeks after the treatment} - \text{EFW one week after the treatment [g]})}{14 \text{ [days]}}$$

iii) Fetal growth rate in the two weeks after the protocol-defined treatment and from the first day of the protocol-defined treatment to birth (%/day).

Fetal growth rate in the two weeks after the protocol-defined treatment (%/day) is calculated using the following formula:

$$\text{Fetal growth rate in the two weeks after the protocol-defined treatment (%/day)} \\ = \frac{\text{EFW two weeks after the treatment} - \text{EFW at the first day of the treatment [g]} \times 100}{\text{EFW at the first day of the treatment [g]} \times 14 \text{ [days]}}$$

and fetal growth rate from the first day of the protocol-defined treatment to birth (%/day) is calculated using the following formula:

$$\text{Fetal growth rate from the first day of the protocol-defined treatment to birth (%/day)} \\ = \frac{\text{Birthweight} - \text{EFW at the first day of the treatment [g]} \times 100}{\text{EFW at the first day of the treatment [g]} \times \text{Days of the treatment [days]}}$$

iv) Fetal head circumference (cm).

The fetal head circumference was measured at the plane of the third ventricle with the thalamus in the central portion and the cavum septi pellucidi visible in the anterior portion.

v) Doppler imaging of umbilical arterial blood flow.

Umbilical arterial blood flow was examined by Doppler ultrasound according to the Society for Maternal-Fetal Medicine (SMFM) Clinical Guidelines.[28]

vi) Deepest amniotic fluid pocket (cm).

The deepest amniotic fluid pocket was measured by transabdominal ultrasonography.

vii) Fetal head circumference, vi) deepest amniotic fluid pocket, and v) doppler imaging of umbilical arterial blood flow are evaluated according to the flow chart as shown below.

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4 **Fetal head circumference, deepest amniotic fluid pocket, and doppler imaging of umbilical**
5 **arterial blood flow evaluation flow chart.**

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5		
	Day of enrollment	1 week after the enrollment	2 weeks after the enrollment	3 weeks after the enrollment	4 weeks after the enrollment	Every two weeks before 36 weeks of GA after visit 5	Every one weeks at or after 37 weeks of GA
Fetal head circumference	•	•	•	•	•	•	•
Deepest amniotic fluid pocket	•	•	•	•	•	•	•
Doppler imaging of umbilical arterial blood flow	•	•	•	•	•	•	•

6
7 vii) Prolongation of GA at birth (days).

8 Prolongation of GA at birth is defined as days from the first day of the protocol-defined
9 treatment to birth.

10 viii) Birth weight (g).

11 Birth weight is defined as the weight of the infant at birth.

12 ix) GA at birth.

13 GA at birth is defined as the gestational age at birth.

14 x) Apgar score.

15 The Apgar score consists of an evaluation of five factors: heart rate, respiratory effort, muscle
16 tone, responsiveness, and color at one minute and five minutes after birth.

17 xi) Umbilical artery pH and base excess values.

18 Umbilical artery pH and base excess is measured at delivery.

19 xii) Incidence rate of pre-eclampsia.

20 Incidence rate of pre-eclampsia is defined as the percentage of enrolled patients who develop
21 pre-eclampsia after the protocol-defined treatment.

22 xiii) Pediatric developmental assessment until 1.5 years of age.

23 Pediatric developmental assessment includes physiological and neurological developmental
24 assessment, and infant complications including cerebral palsy, epilepsy, and death. In the
25 neurodevelopment test in this study, the Kyoto Scale of Psychological Development 2001 was
26 used. Evaluation of neurodevelopment was performed by a pediatric neurologist.

3) Safety endpoints

i) Incidence rate of obstetric complications.

Incidence rate of obstetric complications including hypertensive disorders of pregnancy (HDP) is defined as the percentage of enrolled patients who develop obstetric complications after the protocol-defined treatment.

ii) Perinatal mortality.

Perinatal mortality is defined to include stillbirths (occurring after 22 weeks of gestation) and neonatal deaths (occurring up to 7 days after birth).

iii) Neonatal mortality.

Neonatal mortality is defined as neonatal deaths occurring up to 28 days after birth.

Stopping Criteria

The investigator must discontinue the protocol-defined treatment when certain events prevent continuation of the protocol treatment. These events include the following:

1. The mother has withdrawn her consent to study participation.
2. Certain events prevent continuation of the protocol treatment, which include the following:
 - a) A serious adverse drug reaction to tadalafil has developed.
 - b) The investigator's decision to prioritize other management including termination of the pregnancy instead of continuation of the protocol-defined treatment.
 - c) The investigator's decision that it is inappropriate to continue with the protocol treatment.
 - d) The mother's poor compliance or discontinuation of the protocol treatment.

Criteria for Delivery

In this study, to minimize bias in terms of the timing of delivery, a fetal indication for delivery is established on the basis of the results from the multicenter survey of VLBW infants in Japan using a network database (Figure 1 and Table 1). After registration, all patients will receive the conventional management of FGR according to the guidelines for obstetrical practice in Japan regardless of the treatment arm.[23] Briefly, the conventional management of FGR consists of the evaluation of fetal well-being on ultrasonography, including Doppler imaging of umbilical arterial blood flow, non-stress test, contraction stress test, and biophysical profile scoring depending on GA, to evaluate possible pregnancy termination. The investigator will evaluate the fetal condition and decide timing of delivery referring to Table 1. For other complications such as preterm labor, rupture of the membranes, and hypertensive disorder of pregnancy, the investigator will follow guidelines for obstetric practice in Japan.[23] The investigator must provide a report that explains the reason for termination of the pregnancy on

1 the website of this clinical trial (the Clinical Trial Data Management System).

2 3 **Monitoring Safety during the Fetal Therapy**

4 The investigator must pay close attention to the safety of not only the fetus but also the
5 mother. As shown in the study schedule, the protocol-defined assessments include evaluation of
6 maternal blood pressure and pulse rate, maternal blood and urine tests (blood
7 fibrinogen and anti-thrombin III levels, liver and renal function tests, serum electrolyte levels,
8 and qualitative urine protein excretion), maternal serum placental growth factor (PIGF) and
9 soluble fms-like tyrosine kinase receptor (sFLT-1) levels. Other assessments include adverse
10 events assessed by medical consultation, and antepartum fetal tests consisting of
11 ultrasonography including Doppler imaging of blood flow (umbilical artery, middle cerebral
12 artery, and uterine artery), non-stress test, contraction stress test, and biophysical profile scoring
13 depending on GA. The investigator will enter patients' safety data into the Case Report Form on
14 the website of this clinical trial (the Clinical Trial Data Management System).

15 16 **Safety Evaluation Committee**

17 The Safety Evaluation Committee is responsible for the overall safety of this clinical
18 study. To ensure the safety of the protocol-defined treatment, the Safety Evaluation Committee
19 will review the adverse events of tadalafil treatment. The safety committee had blind access to
20 the data. If a serious adverse event develops, the investigator will provide the Secretariat with
21 the necessary information within 24 hours of its onset, according to the predetermined
22 procedure. The Secretariat then will forward the obtained information without delay to the
23 Safety Evaluation Committee for review. The Safety Evaluation Committee will notify the
24 investigator of the review results. If the adverse event is definitely or probably related to
25 tadalafil treatment, the Ethics Committee in Mie University Hospital or each institute will
26 consider possible termination of this clinical study. Special attention must be paid to the
27 reporting requirements stipulated in the Ethical Guidelines for Clinical Studies (Ministry of
28 Health, Labor, and Welfare in Japan, 2017). Infants will be followed up and evaluated for
29 physiological and neurological development until 1.5 years of age.

30 31 **Sample size**

32 140 fetuses and their mothers.

33 **Rationale for the Target Sample Size**

34 Table 2 shows the summary of the distribution of fetal growth velocity from enrollment to
35 birth in our retrospective study.[22] We estimate that the distribution of fetal growth velocity of
36 this prospective phase II trial will be similar to that of our retrospective study. When the results

of our prospective study are analyzed by Wilcoxon Rank Sum Test and group comparisons, with an α of 0.05, two sided, we will have 90% power to detect a difference if we randomize 62 women per group. Allowing for a 10% drop-out rate, the total sample size required is 140 women.

Table 2. The distribution of fetal growth velocity from enrollment to birth in the retrospective study conducted at Mie University Hospital.

Fetal growth velocity (g/day)	<5	≥5 to <10	≥10 to <15	≥15 to <20	≥20 to <25	≥25
The conventional management group (%)	5.3	10.5	21.1	47.3	15.8	0
The tadalafil group (%)	0	8.3	8.3	50.0	16.7	16.7

Statistical analysis

Analysis is done on all randomized fetuses who receive the protocol-defined treatment at least once, as the full analysis set. All randomised participants with outcome data available will be included in the analyses, which will be performed on an intention-to-treat basis, according to the treatment allocation at randomisation. Analysis ~~per protocol set~~ full analysis set (i.e., removing patients who do not meet the inclusion and exclusion criteria) is done as a secondary analysis population for sensitivity analysis. All outcome measures are presented as summaries of descriptive statistics (mean [SD] or median [minimum, maximum, and interquartile range] for continuous measures, and the numbers and proportions for ordinal and dichotomous measures). Descriptive statistics for the primary endpoint, i.e. fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day), are analyzed for each treatment arm by the Wilcoxon Rank Sum Test and group comparisons. All analyses are performed according to a pre-specified statistical analysis plan. The Data Coordinating Center in Mie University Hospital supports the data management, statistical analysis, and reporting of the study.

Ethics and dissemination

This study was approved by the Institutional Review Board of Mie University Hospital on August 25th, 2016 (No.3041) prior to patient enrollment. The study protocol was also approved by each institutional review board of all participating institutions. This study complies with the Helsinki Declaration. Written informed consent will be obtained from all mothers of fetuses before they are recruited. This trial has been registered in the UMIN Clinical Trials Registry as UMIN000023778 (https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno=R000027132). Our findings will be widely disseminated through conference presentations and peer-reviewed publications.

Participating institutions

Mie University, Showa University, Osaka University, Nagoya University, Mie Chuo Medical Center, Municipal Yokkaichi hospital, Ise Red Cross Hospital, St. Marianna University, Juntendo University, the Jikei University, Toho University, Yokohama City University Medical Center, Kanagawa Children's Medical Center, Ehime University, Hamamatsu University School of Medicine, Osaka Medical College, Niigata University, Showa University Northern Yokohama Hospital, Showa University Koto Toyosu Hospital, Gifu University, University of the Ryukyus, Shiga University, Shinshu University, Ehime Prefectural Central Hospital, Akita University, Tokyo Metropolitan Bokutoh Hospital, Kyorin University, Tokyo Metropolitan Tama Medical Center, Kuwana East Medical Center, Kanazawa University, Nagasaki Medical Center, University of Toyama, Yamaguchi University, Toyota Memorial Hospital, Kainan Hospital, Dokkyo Medical University, Saga Hospital, Kyoto Prefectural University, Toyama Central Prefectural Hospital, Sapporo City General Hospital, Kagoshima University, Mie Prefectural General Medical Center, Kyoto University, Sakakibara Heart Institute, and University of Fukui.

Patient and Public Involvement

Patients with FGR have helped to prioritise this research question through a James Lind Alliance Priority Setting Partnership, which highlighted this as a priority topic. Patients did not involve in the design of this study. Patients were not involved in the recruitment to and conduct of study. Our Results of this study was informed by homepage of Mie University Obstetrics and Gynecology. For randomised controlled trials, there is the no burden of the intervention assessed by patients themselves. Patients and or public were not involved in this trial.

The original protocol is available in *the supplementary file*.

DISCUSSION

This protocol has been already approved by the Institutional Review Board of Mie University Hospital and 39 institutions in Japan. Fetuses with FGR will be enrolled from these institutions. Because fetal growth velocity from the first day of the treatment to birth has been defined as the primary endpoint and fetuses will be randomly assigned in an open-label design, timing of delivery should be made on the basis of similar criteria as much as possible. This study is the first nation-wide intervention study in the field of obstetrics in Japan. We selected an open-label study design with a strict fetal management algorithm on the basis of the results from the multicenter Japanese survey instead of a placebo-controlled design because of operational challenges including low acceptability by pregnant women in Japan. Each

1 participating medical center can provide treatment for fetuses with FGR by board certified
2 members of the Japan Society of Obstetrics and Gynecology, and the investigator will be able to
3 optimally decide timing of delivery according to the guidelines for obstetrical practice in
4 Japan.[23] To make more accurate decisions, a fetal indication for delivery is established in this
5 study on the basis of the results from the multicenter survey in Japan, in which 82 level III
6 perinatal centers, including 8 sites participating in this study, were registered (Table 1).[1] The
7 fetal indication for delivery is divided into three groups depending on infant survival rate in the
8 NICU. Because all patients will undergo antepartum fetal tests consisting of evaluation of fetal
9 well-being by ultrasonography, including Doppler imaging of umbilical arterial blood flow,
10 non-stress test, contraction stress test, and biophysical profile scoring depending on GA
11 according to the Japanese guidelines, the investigator will easily refer to this indication when
12 deciding timing of delivery. This indication will be used to evaluate fetal baseline condition at
13 enrollment as well. We believe that this approach could take advantage of strengths and
14 minimize the possible limitations related to open-label trial features.

15 We retrospectively compared the effect of tadalafil in patients with FGR and
16 demonstrated that both fetal growth velocity from enrollment to birth and birth weight were
17 significantly higher in the tadalafil group than in the conventional management group. The
18 required sample size of this prospective study was estimated based on the results of the
19 retrospective study that used the same primary outcome measure. Since patients with FGR were
20 enrolled in the retrospective study under similar criteria to those in this study, we think that it is
21 reasonable to use the results of the retrospective study for the estimation of sample size.

22
23 **Contributors:** T.U., S.M., M.K, H.T., M.N., K.T., K.O., Y.K., M.E., T. Kimura, T. Kotani, M.N.,
24 A.S., and T.I.: conception of the study. T.U.: writing of the manuscript. S.T., Y.N., M.K., C.M.,
25 and M.N.: providing the biostatistical study design. T.O.: statistical analysis. T. I.: principal
26 Investigator of this trial and the grant holder. All authors have read and approved the final
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28
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32
33 **Competing interests:** None declared.

34
35 **Ethics approval:** The Institutional Review Board of of Mie University Hospital in Augst 25th,
36 2016 (No.3041).

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2 **Data sharing statement:** There is no requirement for data sharing in public research
3 expenditures of our funds, and we are not prepared for data sharing at present. In the future, if
4 the chief researcher receives requests, we will prepare for data sharing to the extent permitted by
5 the Japanese ethics guidelines.

6
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REFERENCES

- 1 Kusuda S, Fujimura M, Sakuma I, *et al.* Morbidity and mortality of infants with very low birth weight in Japan: center variation. *Pediatrics* 2006;**118**:e1130–e1138.
- 2 American College of Obstetricians and Gynecologists. ACOG Practice bulletin no. 134: fetal growth restriction. *Obstet Gynecol* 2013;**121**:1122–33.
- 3 Hui L, Challis D. Diagnosis and management of fetal growth restriction: the role of fetal therapy. *Best Pract Res Clin Obstet Gynaecol* 2008;**22**:139–58.
- 4 Coppage KH, Sun X, Baker RS, *et al.* Expression of phosphodiesterase 5 in maternal and fetal sheep. *Am J Obstet Gynecol* 2005;**193**:1005–10.
- 5 Rotella DP. Phosphodiesterase 5 inhibitors: current status and potential applications. *Nat Rev Drug Discov* 2002;**1**:674–82.
- 6 Wareing M, Myers JE, O'Hara M, *et al.* Effects of a phosphodiesterase-5 (PDE5) inhibitor on endothelium-dependent relaxation of myometrial small arteries. *Am J Obstet Gynecol* 2004;**190**:1283–90.
- 7 Wareing M, Myers JE, O'Hara M, *et al.* Sildenafil citrate (Viagra) enhances vasodilatation in fetal growth restriction. *J Clin Endocrinol Metab* 2005;**90**:2550–5.
- 8 Herraiz S, Pellicer B, Serra V, *et al.* Sildenafil citrate improves perinatal outcome in fetuses from pre-eclamptic rats. *BJOG Int J Obstet Gynaecol* 2012;**119**:1394–402.
- 9 Ramesar SV, Mackraj I, Gathiram P, *et al.* Sildenafil citrate improves fetal outcomes in pregnant, L-NAME treated, Sprague-Dawley rats. *Eur J Obstet Gynecol Reprod Biol* 2010;**149**:22–6.
- 10 Baijnath S, Soobryan N, Mackraj I, *et al.* The optimization of a chronic nitric oxide synthase (NOS) inhibition model of pre-eclampsia by evaluating physiological changes. *Eur J Obstet Gynecol Reprod Biol* 2014;**182**:71–5.
- 11 Nassar AH, Masrouha KZ, Itani H, *et al.* Effects of sildenafil in N^ω-nitro-L-arginine methyl ester-induced intrauterine growth restriction in a rat model. *Am J Perinatol* 2012;**29**:429–34.
- 12 Cross JC, Hemberger M, Lu Y, *et al.* Trophoblast functions, angiogenesis and remodeling of the maternal vasculature in the placenta. *Mol Cell Endocrinol* 2002;**187**:207–12.
- 13 Watson ED, Cross JC. Development of structures and transport functions in the mouse placenta. *Physiol Bethesda Md* 2005;**20**:180–93.
- 14 von Dadelszen P, Dwinnell S, Magee LA, *et al.* Sildenafil citrate therapy for severe early-onset intrauterine growth restriction. *BJOG Int J Obstet Gynaecol* 2011;**118**:624–8.
- 15 Sharp A, Comforth C, Jackson R, *et al.* OC01.05: STRIDER UK: a randomised controlled trial of sildenafil therapy in dismal prognosis early-onset intrauterine growth restriction. [abstract] *Ultrasound Obstet Gynecol* 2017;**50**:3.

- 1 16 Sahni S, Palkar AV, Rochelson BL, *et al.* Pregnancy and pulmonary arterial
2 hypertension: A clinical conundrum. *Pregnancy Hypertens Int J Womens Cardiovasc Health*
3 2015;**5**:157–164.
- 4 17 Ladouceur M, Benoit L, Radojevic J, *et al.* Pregnancy outcomes in patients with
5 pulmonary arterial hypertension associated with congenital heart disease. *Heart Br Card Soc*
6 2017;**103**:287–92.
- 7 18 Daimon A, Kamiya CA, Iwanaga N, *et al.* Management of pulmonary vasodilator
8 therapy in three pregnancies with pulmonary arterial hypertension. *J Obstet Gynaecol Res*
9 2017;**43**:935–938.
- 10 19 Wilkins MR, Wharton J, Grimminger F, *et al.* Phosphodiesterase inhibitors for the
11 treatment of pulmonary hypertension. *Eur Respir J* 2008;**32**:198–209.
- 12 20 Fargue ST, Patterson BE, Bedding AW, *et al.* Tadalafil pharmacokinetics in healthy
13 subjects. *Br J Clin Pharmacol* 2006;**61**:280–288.
- 14 21 Yoshikawa K, Umekawa T, Maki S, *et al.* Tadalafil Improves L-NG-Nitroarginine
15 Methyl Ester-Induced Preeclampsia With Fetal Growth Restriction-Like Symptoms in Pregnant
16 Mice. *Am J Hypertens* 2017;**31**:89–96.
- 17 22 Kubo M, Umekawa T, Maekawa Y, *et al.* Retrospective study of tadalafil for fetal
18 growth restriction: Impact on maternal and perinatal outcomes. *J Obstet Gynaecol Res*
19 2017;**43**:291–297.
- 20 23 Minakami H, Maeda T, Fujii T, *et al.* Guidelines for obstetrical practice in Japan: Japan
21 Society of Obstetrics and Gynecology (JSOG) and Japan Association of Obstetricians and
22 Gynecologists (JAOG) 2014 edition. *J Obstet Gynaecol Res* 2014;**40**:1469–1499.
- 23 24 Kubo M, Tanaka H, Maki S, *et al.* Safety and dose-finding trial of tadalafil administered
24 for fetal growth restriction: A phase-1 clinical study. *J Obstet Gynaecol Res* 2017;**43**:1159–1168.
- 25 25 Cunningham F, Leveno K, Bloom S, *et al.* *Williams Obstetrics, 24e.* Mcgraw-hill 2014.
- 26 26 Parer JT, Ikeda T. A framework for standardized management of intrapartum fetal heart
27 rate patterns. *Am J Obstet Gynecol* 2007;**197**:26–e1.
- 28 27 Shinozuka N. Fetal biometry and fetal weight estimation: JSUM standardization.
29 *Ultrasound Rev Obstet Gynecol* 2002;**2**:156–161.
- 30 28 Berkley E, Chauhan SP, Abuhamad A, *et al.* Doppler assessment of the fetus with
31 intrauterine growth restriction. *Am J Obstet Gynecol* 2012;**206**:300–308.

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5 **FIGURE LEGENDS**

6 **Figure 1. Infant survival rate in the NICU categorized by birth weight and gestational**
7 **week at birth (%).**

8 This figure is established on the basis of the results from the multicenter survey of VLBW
9 infants in Japan using a network database. The survey data included infant survival rates in the
10 NICU, categorized by birth weight and gestational week at birth.[1] The infant survival rate data
11 acquired from the survey were preprocessed with the moving average method and divided into
12 three groups. The first group was defined as “Zone 1” where the infant survival rate in the
13 NICU was less than 60% (highlighted by a red background). The second group was defined as
14 “Zone 2” where the infant survival rate in the NICU ranged from 60 to 95% (highlighted by a
15 yellow background). The third group was defined as “Zone 3” where the infant survival rate in
16 the NICU was 95% or higher (highlighted by a blue background).
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24 **Figure 2. Summary of the study design.**
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Birth weight (g)	1401-1500						96	99	100	99	99	99	99
	1301-1400						94	97	99	99	99	100	99
	1201-1300						98	99	99	99	99	99	100
	1101-1200					96	96	99	100	99	99	99	100
	1001-1100				96	98	98	98	99	99	98	98	98
	901-1000				95	96	97	97	98	99	99	98	97
	801-900			89	91	95	96	96	97	97	98	100	100
	701-800		84	86	90	93	93	95	99	98	94	95	100
	601-700		78	86	90	93	94	93	96	100	100		
	501-600	59	69	80	90	87	93	94	92	87			
	401-500	49	64	71	80	77	80	86	100	71			
	301-400	41	52	51	56	68	67	73	71				
	201-300	18	10	31	33	40							
		22	23	24	25	26	27	28	29	30	31	32	33
		Gestational week at birth											

Figure 1

Figure 1. Infant survival rate in the NICU categorized by birth weight and gestational week at birth (%).

173x177mm (300 x 300 DPI)

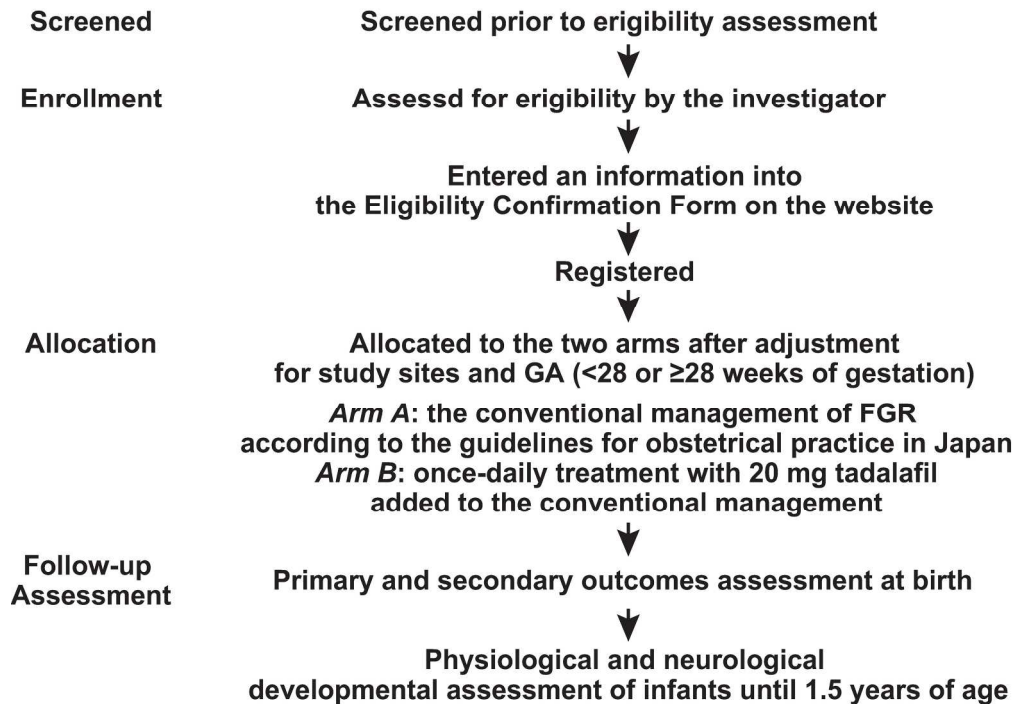


Figure 2

Figure 2. Summary of the study design.

212x193mm (300 x 300 DPI)

Study Protocol

TADAFER II:

A multicenter phase II trial of the efficacy and safety of tadalafil in fetus with early-onset growth restriction.

Trial registration: UMIN Clinical Trials Registry UMIN000023778.

Version 1

Date 25-August-2016

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6 **Contents**

7 SYNOPSIS 3
8
9 1. VOLUNTARY PARTICIPATION AND WITHDRAWAL 5
10
11 2. BACKGROUND AND OBJECTIVES 5
12
13 3. RESEARCH ORGANIZATION AND PARTICIPATING INSTITUTIONS 8
14
15 4. STUDY SUBJECTS AND METHODS..... 10
16
17 5. TREATMENT AND COMPENSATION FOR STUDY-RELATED INJURY 19
18
19 6. STUDY PERIOD AND TARGET SAMPLE SIZE 20
20
21 7. OUTLINE OF THE STUDY PLAN..... 20
22
23 8. ANTICIPATED ADVERSE EVENTS..... 25
24
25 9. POTENTIAL BENEFITS AND RISKS 26
26
27 10. BURDEN OF COST..... 27
28
29 11. INTELLECTUAL PROPERTY RIGHTS 27
30
31 12. ETHICS 27
32
33 13. ADDITIONAL NOTES RELATED TO THE ACT ON THE PROTECTION OF
34 PERSONAL INFORMATION RELATED BY INDEPENDENT ADMINISTRATIVE
35 INSTITUTIONS..... 27
36
37 14. REFERENCE 29
38
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SYNOPSIS

1. Objectives

This multicenter randomized controlled phase II trial has been designed to prospectively evaluate the efficacy and safety of tadalafil treatment in fetus with fetal growth restriction (FGR).

2. Study eligibility

This study will include fetuses and their mothers who meet the following conditions:

- (1) Pregnant women ≥ 20 years.
- (2) Estimated fetal weight (EFW) less than 1.5 standard deviations of the mean EFW for gestational age.
- (3) Gestational age between 20 + 0 and 33 + 6 weeks.
- (4) The expected date of confinement is determined using the criteria of the guidelines for obstetrical practice in Japan (2014).¹
- (5) Singleton pregnancy.
- (6) Signed written informed consent.

3. Treatment

Fetuses with FGR will be randomized to receive either the conventional management of FGR according to the guidelines for obstetrical practice in Japan¹ or once-daily treatment with 20 mg tadalafil added to the conventional management until delivery.

4. Target sample size and duration of the study

Duration of the study: date of ethics approval to February 2021.

Target sample size: 140 singleton fetuses and their mothers.

5. Endpoints

- (1) Primary endpoint: fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day).
- (2) Secondary endpoints
 - 1) Completion rate of the treatment regimen
 - 2) Efficacy endpoints: estimated fetal weight (g), fetal growth velocity in the two weeks after the protocol-defined treatment (g/day), fetal growth velocity in the two weeks after one week of

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6 the protocol-defined treatment (g/day), fetal growth rate (%/day), fetal head circumference (cm),
7 Doppler imaging of umbilical arterial blood flow, deepest amniotic fluid pocket (cm),
8 prolongation of gestational age at birth (days), birth weight (g), gestational age (GA) at birth,
9 Apgar score, umbilical artery pH and base excess values, incidence rate of pre-eclampsia, and
10 pediatric developmental assessment until 1.5 years of age.
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14 3) Safety endpoints: incidence rate of obstetric complications, perinatal mortality, and neonatal
15 mortality.
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18 19 **6. Secretariats**

20
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1. VOLUNTARY PARTICIPATION AND WITHDRAWAL

Participation in this study is on a voluntary basis. Refusal to participate will incur no penalty or loss of benefits to which patients are otherwise entitled to. The subject may withdraw at any time without penalty.

2. BACKGROUD AND OBJECTIVES

Neonatal intensive care has improved over the past few decades, and morbidity among infants, including those who are premature, continues to decline. Premature infants with intrauterine growth restriction, however, still have high mortality and morbidity. The multicenter survey² of very low birth weight (VLBW) infants in Japan conducted by Kusuda and Ikeda *et al.* revealed that mortality in neonatal intensive care units (NICU), of small gestational age (SGA) infants born before 30 weeks gestation, was significantly higher than that of appropriate for gestational age (AGA) infants (unpublished data). To prevent fetal growth restriction (FGR), nutritional and dietary supplementation, bed rest, and aspirin therapy have been investigated, but there is insufficient evidence for the routine indication of any of these treatments.³ There is also no proven therapy to reverse or ameliorate established FGR.⁴

Increases in uteroplacental blood flow during pregnancy via angiogenesis and vasodilation contribute to adequate fetal growth. Vasodilation in the uteroplacental unit is considered to be due to the production and local release of nitric oxide (NO), which stimulates cyclic guanosine monophosphate (cGMP) production.⁵ cGMP is inactivated mainly by phosphodiesterases (PDE), and the predominant PDE isoform present in the vascular smooth muscle is PDE5. Because inhibitors of PDE5, which is a cGMP-specific PDE, exert their pharmacological action by dilating arteries and increasing blood flow, as proven in erectile dysfunction and pulmonary hypertension, recent studies have suggested a potential therapeutic role for PDE5 inhibitors in treating FGR.⁶ Sildenafil, a selective PDE5 inhibitor, has been shown to improve endothelial function in myometrial small arteries removed from women with pre-eclampsia and FGR.^{7,8} However, although sildenafil has been reported to affect maternal hypertension, it has not been shown to affect FGR in studies in FGR model rats induced by L-NG-nitroarginine methyl ester (L-NAME) treatment. In these studies, FGR was not affected by sildenafil except in one report, by Baijnath *et al.*^{9,10,11,12} Baijnath *et al.* demonstrated that L-NAME-induced FGR was improved by sildenafil treatment from 4 days postcoitum (d.p.c.) to

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6 8 d.p.c. but not from 8 d.p.c. to 14 d.p.c.¹¹ Chorioallantoic attachment occurs at 8 d.p.c., and the
7 mature circulatory pattern of maternal blood through the placenta is established by 10 d.p.c. in
8 mouse placenta.^{13,14} In considering the development of fetoplacental circulation in rodents, the
9 effect of sildenafil on fetal growth associated with placental blood flow via an NO-dependent
10 pathway was not manifested. In a clinical study, it was reported that sildenafil was associated
11 with increased fetal abdominal circumference (AC) growth velocity in severe early-onset FGR,
12 but the authors did not report on fetal growth velocity and birth weight.¹⁵ Recently, the
13 STRIDER UK group has found no evidence of a beneficial effect of sildenafil on survival or
14 short-term neonatal outcomes.¹⁶

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21 Tadalafil is also a selective PDE5 inhibitor and has a longer half-life and a more rapid
22 onset of action than sildenafil.⁶ Tadalafil has been used to treat pulmonary hypertension in
23 pregnant women and the Food and Drug Administration in the United States has rated tadalafil
24 as pregnancy category B.¹⁷ When taking sildenafil with a high-fat meal, the time to maximum
25 plasma concentration increases and the peak plasma concentration falls.¹⁸ In contrast, Forgue *et*
26 *al.* reported that food intake had a negligible effect on the bioavailability of tadalafil, and also
27 reported that there was no clinically meaningful effect of gender on tadalafil
28 pharmacokinetics.¹⁹ Our animal experiments demonstrated that tadalafil treatment dilates the
29 maternal blood sinuses in the placenta, which leads to increased placental growth factor (PIGF)
30 production, and contributes to facilitating fetal growth.²⁰ Because tadalafil treatment was started
31 after blood spaces in the placenta were narrowed by L-NAME treatment and elevated urinary
32 excretion of cGMP in these animal experiments, we can safely presume that tadalafil treatment
33 contributes to facilitating fetal growth in the context of the mechanisms associated with NO
34 signaling. In addition, we retrospectively analyzed 11 Japanese singleton pregnant women with
35 FGR who received tadalafil along with conventional management for FGR at Mie University
36 Hospital from July 2015 to February 2016 (tadalafil group).²¹ These women were matched for
37 maternal age, parity, gestational age (GA), and estimated fetal weight at enrollment with 14
38 singleton pregnant women who had received only the conventional management for FGR in
39 2014 (conventional management group). The conventional management for FGR was
40 performed according to the guidelines for obstetric practice in Japan.¹ This retrospective study
41 showed that both fetal growth velocity from enrollment to birth and birth weight were
42 significantly higher in the tadalafil group than in the conventional management group.
43 Moreover, the prevalence of respiratory distress syndrome (RDS) was significantly lower in the
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6 tadalafil group than in the conventional management group. After the retrospective study, we
7 conducted a phase I clinical trial to ensure the safety of tadalafil treatment for FGR.²² There
8 were no serious maternal adverse events for daily tadalafil doses of 10 mg, 20 mg, and 40 mg.
9 More patients who were administered 40 mg tadalafil daily experienced mild adverse events
10 than those administered 10 mg or 20 mg tadalafil daily. In regards to fetal adverse events,
11 intrauterine fetal death occurred in one case. In this case, the pregnant woman was prescribed 40
12 mg tadalafil daily and fetal growth had been progressing at a rate of 22 g/day. At 36 weeks
13 gestation, fetal movement suddenly ceased and a diagnosis of intrauterine fetal death was made.
14 Thereafter, the fetus was delivered vaginally, and velamentous insertion of the umbilical cord
15 was identified. Immediately, the safety evaluation committee investigated the incident's
16 relationship to tadalafil. This committee analyzed the case and concluded that the intrauterine
17 fetal death was due to velamentous insertion of the umbilical cord.²³ We concluded that tadalafil
18 treatment was feasible in pregnant women with FGR.²²

19 Based on the above, we have hypothesized that tadalafil therapy will safely increase the
20 likelihood of increased fetal growth in fetuses with FGR and have designed this multicenter
21 randomized controlled phase II study to establish evidence for fetal therapy with tadalafil. This
22 study, funded by the Japan Agency for Medical Research and Development (AMED), will
23 prospectively evaluate the safety and efficacy of tadalafil in FGR with the participation of major
24 medical centers providing treatment for fetuses with FGR according to the guidelines for
25 obstetrical practice in Japan. Fetuses will be randomized to receive either the conventional
26 management for FGR, according to the guidelines in Japan, or a once-daily treatment with 20
27 mg of tadalafil along with the conventional management, until delivery. Fetal growth velocity
28 from the first day of the protocol-defined treatment to birth (g/day) has been defined as the
29 primary endpoint in this study. To minimize bias in terms of fetal baseline condition and timing
30 of delivery, a fetal indication for delivery is established on the basis of the results from the
31 multicenter survey of VLBW infants in Japan using a network database, in which the 82 level
32 III perinatal centers were registered.² The investigator will evaluate fetal baseline conditions at
33 enrollment and will decide the timing of delivery based on this fetal indication. For other
34 complications such as preterm labor, rupture of the membranes, and hypertensive disorder of
35 pregnancy, the investigator will follow guidelines for obstetric practice in Japan.¹ The
36 investigator will enter the patients' data into the Case Report Form on the website of this
37 clinical trial (the Clinical Trial Data Management System). Infants will be followed up and
38 evaluated for physiological and neurological development until 1.5 years of age.

3. RESEARCH ORGANIZATION AND PARTICIPATING INSTITUTIONS

9	(1) Corresponding	Mie University	Tomoaki Ikeda (Principal Investigator)
10			
11	(2) Collaborator	Showa University	Akihiko Sekizawa
12		Osaka University	Tadashi Kimura
13		Nagoya University	Tomomi Kotani
14		Mie Chuo Medical Center	Yuka Maekawa
15		Municipal Yokkaichi hospital	Kenji Nagao
16		Ise Red Cross Hospital	Tomohisa Kihira
17		St. Marianna University	Nao Suzuki
18		Juntendo University	Satoru Takeda
19		The Jikei University	Aikou Okamoto
20		Toho University	Masahiko Nakata
21		Yokohama City University Medical Center	Shigeru Aoki
22		Kanagawa Children's Medical Center	Hiroshi Ishikawa
23		Ehime University	Takashi Sugiyama
24		Hamamatsu University School of Medicine	Naohiro Kanayama
25		Osaka Medical College	Masahide Ohmichi
26		Niigata University	Takayuki Enomoto
27		Showa University Northern Yokohama Hospital	Kiyotake Ichizuka
28		Showa University Koto Toyosu Hospital	Katsufumi Otsuki
29		Gifu University	Kenichiro Morishige
30		University of the Ryukyu	Yoichi Aoki
31		Shiga University	Takashi Murakami
32		Shinshu University	Tanri Shiozawa
33		Ehime Prefectural Central Hospital	Hiroshi Ochi
34		Akita University	Yukihiro Terada
35		Tokyo Metropolitan Bokutoh Hospital	Hironobu Hyodo
36		Kyorin University	Mitsutoshi Iwashita
37		Tokyo Metropolitan Tama Medical Center	Akira Kohyama
38		Kuwana East Medical Center	Yoshihito Sasaki
39		Kanazawa University	Hiroshi Fujiwara
40		Nagasaki Medical Center	Ichiro Yasuhi

University of Toyama	Shigeru Saito
Yamaguchi University	Norihiro Sugino
Toyota Memorial Hospital	Hidenori Oguchi
Kainan Hospital	Tadashi Sumi
Dokkyo Medical University	Susumu Miyashita
Saga Hospital	Makoto Nomiyama
Kyoto Prefectural University	Jo Kitawaki
Toyama Central Prefectural Hospital	Hiroshi Funamoto
Sapporo City General Hospital	Kazuhiko Okuyama
Kagoshima University	Hiroaki Kobayashi
Mie Prefectural General Medical Center	Hirohiko Tanaka
Kyoto University	Masaki Mandai
Sakakibara Heart Institute	Shinji Katsuragi
University of Fukui	Yoshio Yoshida

(3) Safety Evaluation Committee

The Safety Evaluation Committee is independent from research organization, and responsible for the overall safety of this clinical study. To ensure the safety of the protocol-defined treatment, the Safety Evaluation Committee will review adverse events of tadalafil. The Safety Evaluation Committee consists of Dr. Makoto Maeda (Board Certified Member of the Japan Society of Obstetrics and Gynecology) and Dr. Yoshiaki Miyake (Board Certified Member of the Japan Society of Obstetrics and Gynecology).

(4) Protocol Evaluation Committee

The Protocol Evaluation Committee is an organization of the execution of this study. All experimental protocols are evaluated and approved by the Protocol Evaluation Committee.

(5) Data Coordinating Center at the Clinical Research Support Center in Mie University Hospital

This center supports the data management, and statistical analysis and reporting of the study. This consists of Dr. Masakatsu Nishikawa (chairperson), Ms. Yuki Nishimura (data manager), and Dr. Toru Ogura (statistics).

(6) Secretariats

Dr. Takashi Umekawa, Dr. Shintaro Maki, and Dr. Michiko Kubo.

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4. STUDY SUBJECTS AND METHODS

(1) Study Sites and Subjects

1) Study Sites

This is a multicenter randomized controlled phase II trial, in which the Clinical Research Support Center in Mie University Hospital serves as the data center. Since this trial has been designed to prospectively evaluate the efficacy and safety of tadalafil treatment in FGR, fetuses will be randomized to receive either the conventional management for FGR, according to the guidelines in Japan¹ or a once-daily treatment with 20 mg of tadalafil along with the conventional management at the data center. Case registration requires the approval of the Ethics Committee. The following institutions will participate in this clinical trial:

Mie University, Showa University, Osaka University, Nagoya University, Mie Chuo Medical Center, Municipal Yokkaichi hospital, Ise Red Cross Hospital, St. Marianna University, Juntendo University, the Jikei University, Toho University, Yokohama City University Medical Center, Kanagawa Children's Medical Center, Ehime University, Hamamatsu University School of Medicine, Osaka Medical College, Niigata University, Showa University Northern Yokohama Hospital, Showa University Koto Toyosu Hospital, Gifu University, University of the Ryukyus, Shiga University, Shinshu University, Ehime Prefectural Central Hospital, Akita University, Tokyo Metropolitan Bokutoh Hospital, Kyorin University, Tokyo Metropolitan Tama Medical Center, Kuwana East Medical Center, Kanazawa University, Nagasaki Medical Center, University of Toyama, Yamaguchi University, Toyota Memorial Hospital, Kainan Hospital, Dokkyo Medical University, Saga Hospital, Kyoto Prefectural University, Toyama Central Prefectural Hospital, Sapporo City General Hospital, Kagoshima University, Mie Prefectural General Medical Center, Kyoto University, Sakakibara Heart Institute, and University of Fukui.

2) Subjects and Diagnostic Methods

All patients have to meet all inclusion criteria without violating any of the exclusion criteria listed below. All subjects will be followed-up until the end of the study.

Inclusion Criteria

(1) Pregnant women ≥ 20 years.

- (2) EFW less than 1.5 standard deviations of the mean EFW for GA.
- (3) GA between 20 + 0 and 33 + 6 weeks.
- (4) The expected date of confinement is determined using the criteria of the guidelines for obstetrical practice in Japan (2014).
- (5) Singleton pregnancy.
- (6) Signed written informed consent.

Exclusion Criteria

- (1) A result from the antepartum fetal tests, done at enrollment, which indicates that delivery should be attempted.
- (2) A history of allergy to tadalafil.
- (3) Concurrent medications that interact adversely with tadalafil.
- (4) Contraindication of tadalafil treatment due to renal disease.
- (5) Contraindication of tadalafil treatment due to liver disease.
- (6) Contraindication of tadalafil treatment due to uncontrolled arrhythmia, hypertension (BP >170/100 mmHg), and hypotension (BP <80/40 mmHg).
- (7) Fetus with suspected chromosomal disorder and/or multiple congenital anomalies.
- (8) Contraindication of tadalafil treatment due to retinitis pigmentosa, coagulation defect, active gastric and/or intestinal ulcer, and venous obstructive disease.
- (9) The investigator decides to entry inappropriate.

Rationale for Eligibility Criteria

- When diagnosed as FGR, the mean EFW for GA but not the mean birthweight for GA should be used, and the estimated date of confinement using fetal measurements obtained during the early stage of pregnancy should be confirmed according to the guidelines for obstetrical practice in Japan (2014) in Inclusion Criteria Nos. 2 and 4.¹
- The lower age limit (20 weeks gestation) of Inclusion Criterion No. 3 is determined referring to the previous study protocol about the treatment for FGR.²⁴ The upper limit of <34 weeks gestation is based on infant survival rate in the NICU categorized by birth weight and gestational week at birth from the Japanese neonatal research network database (<http://nponrn.umin.jp/index.html> Japanese-only website), in which indicates that treatments are prioritized over elective delivery (Figure 1).

from 60 to 95%. The third group was defined as “Zone 3” where the infant survival rate in the NICU was 95% or higher. All patients in our study will undergo antepartum fetal tests consisting of the evaluation of fetal well-being by ultrasonography, including Doppler imaging of umbilical arterial blood flow, non-stress test, contraction stress test, and biophysical profile scoring depending on GA, to evaluate possible pregnancy termination by the investigator at enrollment (Table 1. Exclusion Criterion No. 1).

Table 1. A fetal indication for delivery in the TADAFER II study.^{1,23,25}

Infant survival rate in the NICU (See Figure 1)	
Zone 1	Decide timing of delivery depending on available therapeutic measures at NICU in each institute.
Zone 2	Consider delivery if at least one of three findings is made, but place give high priority on the determination by the investigators. <ol style="list-style-type: none"> 1. Reversed umbilical artery blood flow during diastole. 2. Score less than 4 on the fetal biophysical profile score. 3. Feat heart rate patterns in the orange or red category for more than 30 minutes.²⁵
Zone 3	Consider delivery if at least one of five findings is made, but place give high priority on the determination by the investigators. <ol style="list-style-type: none"> 1. Reversed or absent umbilical artery blood flow during diastole. 2. Score less than 4 on the fetal biophysical profile score. (Score less than 6 on the fetal biophysical profile score if oligohydramnios is present.) 3. Feat heart rate patterns in the orange or red category for more than 30 minutes.²⁵ 4. Positive contraction stress test. 5. Impaired fetal head circumference growth for more than 2 weeks.

- Patients who have contraindications for tadalafil treatment will be excluded (Exclusion Criteria from No.2 to No.7).
- Regarding exclusion criteria No.9, this study excludes mothers with mental or psychiatric problems, since poor judgment capabilities that are often associated with such conditions may not be compatible with Inclusion Criterion No. 6.

(2) Study Design

This study is a multicenter randomized controlled phase II trial.

(3) Methods

In this multicenter clinical study, each study site will obtain ethics approval of the protocol before its implementation.

Registration

This study protocol defines all the procedures and schedules that the investigator must abide by to complete this clinical study, including patient selection and registration, fetal treatment of FGR, and follow-up (Figure 2).

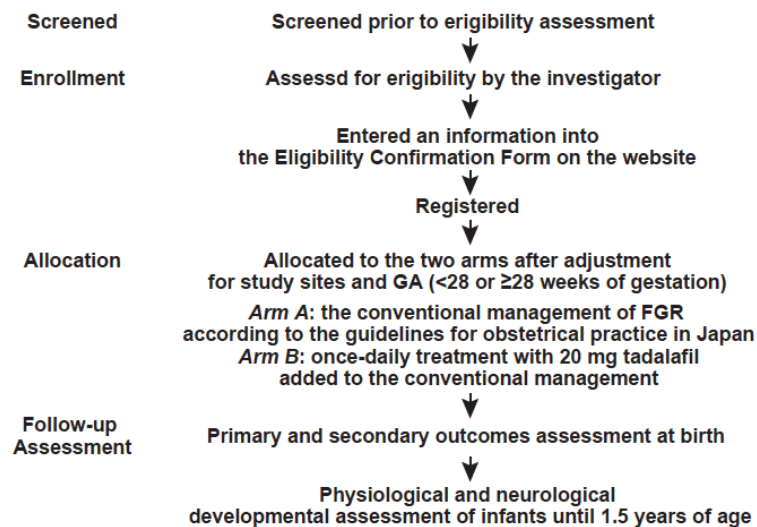


Figure 2. Summary of the study design.

The Clinical Research Support Center in Mie University Hospital will provide data center services including data management and patient registration. Patients that satisfy all inclusion criteria and do not meet any of the exclusion criteria will be eligible for inclusion in the study. Individual study sites will be responsible for guiding potential participants through the informed consent process, including patients who have been referred to them for treatment purposes. The investigator will enter an eligible patient's information into the Eligibility Confirmation Form on the website of this clinical trial (the Clinical Trial Data Management System: Japanese-only website). The data management system will check the contents of the form before registering the patient. For patients who meet all inclusion criteria without violating any of the exclusion criteria listed above, the data management system will register and allocate them to the two arms in an allocation ratio of 1:1, one group receiving the conventional management of FGR according to the guidelines for obstetrical practice in Japan,¹ and the other receiving once-daily treatment with 20 mg tadalafil added to conventional management after adjustment for study

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6 sites and GA (<28 or ≥28 weeks of gestation). The investigators are blinded to the allocation
7 algorithm. Enrolled participants will receive fetal therapy within 7 days of registration. The
8 investigator will enter the patients' data into the Case Report Form on the website of this
9 clinical trial (the Clinical Trial Data Management System).
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12 The corresponding researcher at Mie University will be responsible for the management of this
13 study (patient registration, data management, and coordination with the study-related
14 committees and the Clinical Research Support Center in Mie University Hospital). The
15 corresponding researcher will also be responsible for the research administration, scheduling,
16 documentation, and safety information management. The Safety Evaluation Committee will
17 assume responsibility for the safety of this study. The Clinical Research Support Center in Mie
18 University Hospital will provide technical support from the planning to the completion of this
19 clinical study. Its Data Management Department will manage the study data in cooperation with
20 the corresponding researcher and secretariats, and its Statistics Department will provide
21 statistical support to facilitate the efficacy evaluation. The Protocol Evaluation Committee is an
22 organization of the execution of this study. All experimental protocols are evaluated and
23 approved by the Protocol Evaluation Committee.
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34 **UMIN Clinical Trials Registry UMIN000023778.**

35 36 37 **Fetal Treatment Protocol**

38 The investigator will provide the fetal therapy as described below.

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40 *Arm A:* the conventional management of FGR according to the guidelines for obstetrical
41 practice in Japan.¹ Briefly, the conventional management of FGR consists of evaluation of fetal
42 well-being on ultrasonography, including Doppler imaging of umbilical arterial blood flow,
43 non-stress test, contraction stress test, and biophysical profile scoring depending on GA to
44 evaluate possible pregnancy termination.
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48 *Arm B:* once-daily treatment with 20 mg tadalafil added to the conventional management until
49 delivery.
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52 The investigators are blinded to the allocation algorithm. Enrolled participants will receive fetal
53 therapy within 7 days of registration.
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55 56 **Rationale for Dose Selection**

57 Tadalafil was approved for treatment of erectile dysfunction (ED) in July 2007 in Japan.
58 Nishiuma S *et al.* reported the results from a post marketing surveillance study on tadalafil, with
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6 a primary goal of confirming the safety and effectiveness of tadalafil in Japanese patients with
7 ED in routine clinical practice. 86.7 % of the participants in the surveillance study were
8 prescribed 10mg or 20mg tadalafil daily.²⁶ We referred the results of adverse events in the
9 surveillance study and determined the dose of tadalafil in our retrospective study, in which three
10 pregnant women (27.3%) were prescribed 10 mg tadalafil daily and eight pregnant women
11 (72.7%) were prescribed 20 mg daily.²¹ In our phase I study, more patients who were
12 administered 40 mg tadalafil daily experienced adverse events than those administered 10 mg or
13 20 mg tadalafil daily, but we found that there were no serious maternal adverse events.²² Finally,
14 the minimum required sample size was estimated based on the results of our retrospective study.
15 Taken together, the tadalafil dosage (once-daily treatment with 20 mg) was set in this study.
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24 **Stopping Criteria**

25 The investigator must discontinue the protocol-defined treatment when certain events prevent
26 continuation of the protocol treatment. These events include the following:
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- 29 1. The mother has withdrawn her consent to study participation.
- 30 2. Certain events prevent continuation of the protocol treatment, which include the following:
 - 31 a) A serious adverse drug reaction to tadalafil has developed.
 - 32 b) The investigator's decision to prioritize other management including termination of the
 - 33 pregnancy instead of continuation of the protocol-defined treatment.
 - 34 c) The investigator's decision that it is inappropriate to continue with the protocol treatment.
 - 35 d) The mother's poor compliance or discontinuation of the protocol treatment.

36 Fetuses in whom the fetal therapy was prematurely discontinued as per the Stopping Criteria
37 will receive scheduled examinations and other assessments to the extent possible. If the mother
38 withdraws her consent to study participation, she and her fetus will be removed from the study.
39 If the fetal therapy is prematurely discontinued due to a serious adverse drug reaction to
40 tadalafil, scheduled subsequent examinations and other assessments should be continued to the
41 extent possible and the investigator should provide the patient experiencing an adverse event
42 with the most appropriate therapeutic measures available. If a registered mother or her fetus is
43 found to have been non-conformant to the eligibility criteria, poor compliance and dropping out
44 with the protocol treatment, the mother or fetus will be categorized as noncompliant.
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57 **Criteria for Delivery**

58 In this study, to minimize bias in terms of the timing of delivery, a fetal indication for delivery
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6 is established on the basis of the results from the multicenter survey of VLBW infants in Japan
7 using a network database (Figure 1 and Table 1). After registration, all patients will receive the
8 conventional management of FGR according to the guidelines for obstetrical practice in Japan
9 regardless of the treatment arm.¹ Briefly, the conventional management of FGR consists of the
10 evaluation of fetal well-being on ultrasonography, including Doppler imaging of umbilical
11 arterial blood flow, non-stress test, contraction stress test, and biophysical profile scoring
12 depending on GA, to evaluate possible pregnancy termination. The investigator will evaluate
13 the fetal condition and decide timing of delivery referring to Table 1. For other complications
14 such as preterm labor, rupture of the membranes, and hypertensive disorder of pregnancy, the
15 investigator will follow guidelines for obstetric practice in Japan.¹ The investigator must
16 provide a report that explains the reason for termination of the pregnancy on the website of this
17 clinical trial (the Clinical Trial Data Management System).
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27 **Monitoring Safety during the Fetal Therapy**

28 The investigator must pay close attention to the safety of not only the fetus but also the mother.
29 As shown in the study schedule, the protocol-defined assessments include evaluation of
30 maternal blood pressure and pulse rate, maternal blood and urine tests (blood
31 fibrinogen and anti-thrombin III levels, liver and renal function tests, serum electrolyte levels,
32 and qualitative urine protein excretion), maternal serum placental growth factor (PIGF) and
33 soluble fms-like tyrosine kinase receptor (sFLT-1) levels. Other assessments include adverse
34 events assessed by medical consultation, and antepartum fetal tests consisting of
35 ultrasonography including Doppler imaging of blood flow (umbilical artery, middle cerebral
36 artery, and uterine artery), non-stress test, contraction stress test, and biophysical profile scoring
37 depending on GA. The investigator will enter patients' safety data into the Case Report Form on
38 the website of this clinical trial (the Clinical Trial Data Management System).
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49 **Safety Evaluation Committee**

50 The Safety Evaluation Committee is responsible for the overall safety of this clinical study. To
51 ensure the safety of the protocol-defined treatment, the Safety Evaluation Committee will
52 review the adverse events of tadalafil treatment. If a serious adverse event develops, the
53 investigator will provide the Secretariat with the necessary information within 24 hours of its
54 onset, according to the predetermined procedure. The Secretariat then will forward the obtained
55 information without delay to the Safety Evaluation Committee for review. The Safety
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6 Evaluation Committee will notify the investigator of the review results. If the adverse event is
7 definitely or probably related to tadalafil treatment, the Ethics Committee in Mie University
8 Hospital or each institute will consider possible termination of this clinical study. Special
9 attention must be paid to the reporting requirements stipulated in the Ethical Guidelines for
10 Clinical Studies (Ministry of Health, Labor, and Welfare in Japan, 2017). Infants will be
11 followed up and evaluated for physiological and neurological development until 1.5 years of
12 age.
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19 **Note for New Participating Study Sites**

20 This multicenter study is open to new study sites. It is desirable that study sites cooperate with
21 each other. Agreement on this inter-institutional cooperation is a prerequisite for participation in
22 this clinical study. Case registration requires the approval of the Ethics Committee in each
23 institute.
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5. TREATMENT AND COMPENSATION FOR STUDY-RELATED INJURY

Based on our previous studies, we do not expect that serious adverse events will occur frequently in this study.²² However, the investigator may encounter such adverse events as those mentioned in Section 8: Anticipated Adverse Events. The investigator must report adverse drug reactions to the Minister of Health, Labour and Welfare as provided in the Pharmaceuticals and Medical Devices Act. The investigator must also report any serious adverse events without delay to the head of his or her institution, who will in turn forward the information to the Secretariat. The Secretariat will inform the participating study sites of all reported serious adverse events, irrespective of whether expected or unexpected. The Safety Evaluation Committee will review serious adverse event reports and make recommendations to the Principal Investigator, as appropriate. More specifically, the Safety Evaluation Committee will review the information on a serious adverse event that the investigator forwarded as per the predetermined procedure to the Secretariat within 24 hours of its onset. The Safety Evaluation Committee will notify the review results to the investigator. If the adverse event is definitely or probably related to tadalafil treatment, the Ethics Committee in Mie University Hospital or each institute will consider possible termination of this clinical study. Special attention must be paid to the reporting requirements stipulated in the Ethical Guidelines for Clinical Studies (Ministry of Health, Labor, and Welfare in Japan, 2017).

According to the provisions of the Ethical Guidelines for Clinical Studies (Ministry of Health, Labour and Welfare in Japan, 2017), the study site will inform the Ministry of Health, Labour and Welfare of unexpected adverse events whose study causality cannot be denied. The Ministry of Health, Labour and Welfare will announce reported serious adverse drug reactions to the public at regular intervals. The study site must provide the patient experiencing an adverse event with the most appropriate therapeutic measures available. In this clinical study, maternal complications associated with the protocol-defined treatment have been covered by liability insurance. However, because fetal complications associated with the protocol-defined treatment have not been covered by liability insurance, the investigator must describe this issue in the informed consent document. The corresponding researcher at Mie University is responsible for dealing with inquiries from participating study sites. In case of an accident, the corresponding researcher will consult the Ethics Committee in Mie University for guidance. This study will comply with the reporting requirements provided in the Ethical Guidelines for Clinical Studies (Ministry of Health, Labour and Welfare in Japan, 2017).

6. STUDY PERIOD AND TARGET SAMPLE SIZE

(1) Study Period

The planned study period is from date of ethics approval to February 2021. The Patient Registration Period will last until December 2018. The children's outcome will be followed up for 1.5 years after birth. Data collected by the end of the Neonatal Evaluation Period will be subjected to statistical analysis.

Patient Registration Period: date of ethics approval to December 2018.

Children's Outcome Follow-up Period: 1.5 years after the last birth

(2) Target Sample Size

140 fetuses and their mothers

Rationale for the Target Sample Size

Table 2 shows the summary of the distribution of fetal growth velocity from enrollment to birth in our retrospective study.²¹ We estimate that the distribution of fetal growth velocity of this prospective phase II trial will be similar to that of our retrospective study. When the results of our prospective study are analyzed by Wilcoxon Rank Sum Test and group comparisons, with an α of 0.05, two sided, we will have 90% power to detect a difference if we randomize 62 women per group. Allowing for a 10% drop-out rate, the total sample size required is 140 women.

Table 2. The distribution of fetal growth velocity from enrollment to birth in the retrospective study conducted at Mie University Hospital.

Fetal growth velocity (g/day)	<5	≥5 to <10	≥10 to <15	≥15 to <20	≥20 to <25	≥25
The conventional management group (%)	5.3	10.5	21.1	47.3	15.8	0
The tadalafil group (%)	0	8.3	8.3	50.0	16.7	16.7

7. OUTLINE OF THE STUDY PLAN

1. The investigator will register patients with the Clinical Trial Data Management System according to the procedure defined above.
2. The Clinical Trial Data Management System will register and allocate them to the two arms in an allocation ratio of 1:1, one group receiving the conventional management of FGR according to the guidelines for obstetrical practice in Japan,¹ and the other receiving once-daily treatment with 20 mg tadalafil added to conventional management after adjustment for study sites and GA (<28 or ≥28 weeks of gestation).

3. The investigator will conduct the protocol-defined treatment. The Stopping Criteria and the Criteria for Delivery are explained in detail above.

4. Timing and Methods of Evaluation

The investigator will evaluate the variables listed below according to the study schedule. The investigator will use the Case Report Form on the website of this clinical trial (the Clinical Trial Data Management System).

5. Variables

The following safety and efficacy variables will be statistically analyzed:

Variables

(1) Maternal and Fetal

i) Signs and symptoms

Headache, vertigo, flushing, epistaxis, palpitations, anorexia, dyspepsia, diarrhea, nausea, myalgia, arthralgia, dyspnea, and fetal movement counting.

ii) Maternal vital signs

Blood pressure and pulse rate.

iii) Maternal blood and urine test

Complete blood count, blood fibrinogen and anti-thrombin 3 levels, liver and renal function tests, serum electrolyte levels, qualitative urine protein excretion, maternal serum placental growth factor (PIGF), and soluble fms-like tyrosine kinase receptor (sFLT-1) levels.

iv) Fetal ultrasound examination

Estimated fetal weight (g), fetal head circumference (cm), deepest amniotic fluid pocket (cm), Doppler imaging of blood flow (umbilical artery, middle cerebral artery, and uterine artery)

v) Obstetrics

Onset of obstetrical complications including hypertensive disorder of pregnancy (HDP), treatment for obstetrical complications, indication for delivery, mode of delivery, and placental weight.

vi) Compliance of tadalafil treatment (arm B only).

vi) Adverse events

(2) Neonatal

i) GA at birth.

ii) Physical development

Body weight, height, head circumference, and percentile of birth weight for GA and sex

iii) Apgar score

iv) Clinical laboratory testing

Umbilical artery pH and base excess values

v) Admission in the NICU

vi) Neonatal complications

Respiratory distress syndrome (RDS), pulmonary hemorrhage, neonatal pulmonary hypertension, neonatal chronic lung disease, symptomatic patent ductus arteriosus (PDA), late-onset circulatory dysfunction, intraventricular hemorrhage, periventricular leukomalacia, hypoxic-ischemic encephalopathy, sepsis, necrotizing enterocolitis, gastroesophageal reflux, meconium plug syndrome, retinopathy of prematurity (ROP), anemia of prematurity, auditory disorder (abnormal auditory brainstem response results), congenital abnormality, death, and others.

(3) Pediatric

Physiological and neurological developmental assessment until 1.5 years of age, infant complications including cerebral palsy and epilepsy, and death.

Study Endpoints

(1) Primary endpoint

Fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day).

The primary endpoint is fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day), and is calculated using the following formula:

$$\text{Fetal growth velocity (g/day)} = \frac{\text{Birthweight} - \text{EFW at the first day of the treatment [g]}}{\text{Days of the treatment [days]}}$$

Rationale for the primary endpoint

Our primary hypothesis is that tadalafil therapy will increase the likelihood of increased fetal growth velocity in fetuses with FGR. Taking into account the results of our retrospective study demonstrating that tadalafil treatment increased fetal growth velocity (g/day), which was the primary outcome of the retrospective study, and decreased the incidence of RDS, an improvement in fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day) is an important indicator of the therapeutic benefits for fetuses with FGR.²¹

(2) Secondary endpoints

1) Completion rate of the treatment regimen.

Completion rate of the treatment regimen is defined as the percentage of enrolled patients who receive the protocol-defined treatment for more than 7 days.

2) Efficacy endpoints.

i) Estimated fetal weight (g).

Estimated fetal weight (EFW) is calculated using the following formula:²⁷

$$\text{EFW (g)} = 1.07 \times (\text{biparietal diameter: BPD})^3 + 0.3 \times (\text{abdominal circumference: AC})^2 \times (\text{femur length: FL})$$

ii) Fetal growth velocity in the two weeks after the protocol-defined treatment and in the two weeks after one week of the protocol-defined treatment (g/day).

Fetal growth velocity in the two weeks after the protocol-defined treatment (g/day) is calculated using the following formula:

$$\begin{aligned} & \text{Fetal growth velocity in the two weeks after the protocol – defined treatment (g/day)} \\ &= \frac{(\text{EFW two weeks after the treatment} - \text{EFW at the first day of the treatment [g]})}{14 \text{ [days]}} \end{aligned}$$

and fetal growth velocity in the two weeks after one week of the protocol-defined treatment (g/day) is calculated using the following formula:

$$\begin{aligned} & \text{Fetal growth velocity in the two weeks after one week of the treatment (g/day)} \\ &= \frac{(\text{EFW three weeks after the treatment} - \text{EFW one week after the treatment [g]})}{14 \text{ [days]}} \end{aligned}$$

iii) Fetal growth rate in the two weeks after the protocol-defined treatment and from the first day of the protocol-defined treatment to birth (%/day).

Fetal growth rate in the two weeks after the protocol-defined treatment (%/day) is calculated using the following formula:

$$\begin{aligned} & \text{Fetal growth rate in the two weeks after the protocol-defined treatment (%/day)} \\ &= \frac{\text{EFW two weeks after the treatment} - \text{EFW at the first day of the treatment [g]}}{\text{EFW at the first day of the treatment [g]}} \times 100 \\ &= \frac{\hspace{10em}}{14 \text{ [days]}} \end{aligned}$$

and Fetal growth rate from the first day of the protocol-defined treatment to birth (%/day) is calculated using the following formula:

$$\begin{aligned} & \text{Fetal growth rate from the first day of the protocol-defined treatment to birth (%/day)} \\ &= \frac{\text{Birthweight} - \text{EFW at the first day of the treatment [g]}}{\text{EFW at the first day of the treatment [g]}} \times 100 \\ &= \frac{\hspace{10em}}{\text{Days of the treatment [days]}} \end{aligned}$$

iv) Fetal head circumference (cm).

The fetal head circumference was measured at the plane of the third ventricle with the thalamus

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6 in the central portion and the cavum septi pellucidi visible in the anterior portion.

7 v) Doppler imaging of umbilical arterial blood flow.

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9 Umbilical arterial blood flow was examined by Doppler ultrasound according to the Society for
10 Maternal-Fetal Medicine (SMFM) Clinical Guideline.²⁸

11
12 vi) Deepest amniotic fluid pocket (cm).

13
14 The deepest amniotic fluid pocket was measured by transabdominal ultrasonography.

15
16 vii) Prolongation of gestational age at birth (days).

17
18 Prolongation of gestational age at birth is defined as days from the first day of the
19 protocol-defined treatment to birth.

20
21 viii) Birth weight (g).

22
23 Birth weight is defined as the weight of the infant at birth.

24
25 ix) GA at birth.

26
27 GA at birth is defined as the gestational age at birth.

28
29 x) Apgar score.

30
31 The Apgar score consists of an evaluation of five factors: heart rate, respiratory effort, muscle
32 tone, responsiveness and color at one minute and five minutes after birth.

33
34 xi) Umbilical artery pH and base excess values.

35
36 Umbilical artery pH and base excess is measured at delivery.

37
38 xii) Incidence rate of pre-eclampsia.

39
40 Incidence rate of pre-eclampsia is defined as the percentage of enrolled patients who develop
41 pre-eclampsia after the protocol-defined treatment.

42
43 xiii) Pediatric developmental assessment until 1.5 years of age.

44
45 Pediatric developmental assessment includes physiological and neurological developmental
46 assessment, and infant complications including cerebral palsy, epilepsy, and death.

47 48 3) Safety endpoints

49
50 i) Incidence rate of obstetric complications.

51
52 Incidence rate of obstetric complications including HDP is defined as the percentage of enrolled
53 patients who develop obstetric complications after the protocol-defined treatment.

54
55 ii) Perinatal mortality.

56
57 Perinatal mortality is defined to include stillbirths (occurring after 22 weeks of gestation) and
58 neonatal deaths (occurring up to 7 days after birth).

59
60 iii) Neonatal mortality.

Neonatal mortality is defined as neonatal deaths occurring up to 28 days after birth.

(3) Statistics

Analysis is done on all randomized fetuses who receive the protocol-defined treatment at least once, as the full analysis set. Analysis per protocol set (i.e., removing patients who do not meet the inclusion and exclusion criteria) is done as a secondary analysis population for sensitivity analysis. All outcome measures are presented as summaries of descriptive statistics (mean [SD] or median [minimum and maximum] for continuous measures, and the numbers and proportions for ordinal and dichotomous measures). Descriptive statistics for the primary endpoint, i.e. fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day), are analyzed for each treatment arm by the Wilcoxon Rank Sum Test and group comparisons. All analyses are performed according to a pre-specified statistical analysis plan. The Data Coordinating Center in Mie University Hospital supports the data management, statistical analysis, and reporting of the study.

8. ANTICIPATED ADVERSE EVENTS

Because we have already demonstrated in phase I clinical trial that tadalafil treatment was feasible in pregnant women with FGR,²² tadalafil treatment for FGR can be administered with relative safety and ease. Yet, this therapy may give rise to unexpected adverse events, given the limited clinical experience with this approach and exposure of healthy mothers without pulmonary hypertension to tadalafil. The investigator must fully inform prospective participants of such possibility and administer the fetal therapy with careful attention and monitoring. Adverse reactions to tadalafil divided into the four groups by the frequency (Very common [$\geq 1/10$], common [$\geq 1/100$ to $< 1/10$], uncommon [$\geq 1/1,000$ to $< 1/100$], and not known [cannot be estimated from the available data]) described in the product information of tadalafil (ADCIRCA[®] 20 mg tablets) are shown below:²⁹

- Very common ($\geq 1/10$)
Headache, flushing, nasopharyngitis, nausea, dyspepsia, myalgia, neck pain, and pain in extremity.
- Common ($\geq 1/100$ to $< 1/10$)
Hypersensitivity reactions*, syncope, migraine*, blurred vision, palpitations*^{***}, hypotension, epistaxis, vomiting, gastroesophageal reflux, rash, increased uterine bleeding**, facial oedema, and chest pain***.
- Uncommon ($\geq 1/1,000$ to $< 1/100$)

Seizures*, transient amnesia*, tinnitus, Sudden cardiac death****, Tachycardia****, hypertension, urticaria*, hyperhidrosis*, haematuria, priapism*, penile haemorrhage, and haematospermia

- Not known (cannot be estimated from the available data)
Angioedema, stroke***, non-arteritic anterior ischemic optic neuropathy, retinal vascular occlusion, visual, field defect, sudden hearing loss, unstable angina pectoris, ventricular arrhythmia, myocardial infarction***, Stevens-Johnson Syndrome, exfoliative dermatitis, and prolonged erections.

* The adverse reactions have been included in the table as a result of postmarketing or clinical study data from the use of tadalafil in the treatment of erectile dysfunction; and in addition, the frequency estimates are based on only 1 or 2 patients experiencing the adverse reaction in the pivotal placebo controlled study of ADCIRCA®.

** Clinical non-Medical Dictionary for Regulatory Activities (MedDRA) term to include reports of abnormal/excessive menstrual bleeding, conditions such as menorrhagia, metrorrhagia, menometrorrhagia, or vaginal hemorrhage.

***Most of the patients in whom these events have been reported had pre-existing cardiovascular risk factors.

9. POTENTIAL BENEFITS AND RISKS

(1) Benefits

Potential benefits of this study include cure or improvement in FGR.

(2) Risks

Maternal exposure to tadalafil is inevitable in patients allocated tadalafil treatment arm. Therefore, precautions must ensure the safety of both the mother and the fetus. Specific descriptions of such risks have been described in Section 8: Anticipated Adverse Events. To control for such risks, this study has stipulated an array of tests, such as hematology, serum chemistry, medical consultation, and antepartum fetal tests consisting of ultrasonography including Doppler imaging of blood flow (umbilical artery, middle cerebral artery, and uterine artery), non-stress test, contraction stress test, and biophysical profile scoring depending on GA. In the event of an adverse drug reaction, the investigator will immediately take appropriate measures, possibly including early withdrawal from the study. The investigator must prioritize maternal safety over fetal therapy. If the mother develops an adverse drug reaction, it will be treated under liability insurance and / or the national health insurance scheme.

10. BURDEN OF COST

This research was supported by by the Japan Agency for Medical Research and Development (AMED). This fund will be paid for items related to research (purchasing cost for tadalafil, data management, storage, analysis, etc.) other than medical examination. Medical examination expenses are covered by the national health insurance scheme.

11. INTELLECTUAL PROPERTY RIGHTS

Any intellectual property rights that may arise from this clinical study shall be exclusively owned by the TADAFER study group. The corresponding researcher and the joint researchers report no conflicts of interest related to this clinical study or to their organizations.

12. ETHICS

This clinical study focuses on prenatal treatment, and its protocol has been developed according to the Ethical Guidelines for Clinical Studies (Ministry of Health, Labour and Welfare in Japan, 2017). Before the start of this clinical study, the corresponding researcher will explain its objectives and outline them fully to the participating site investigators. We believe that application of the guideline requirements to the mother who consents to participate in this study will ensure that her fetus is also protected by the ethical principles of the guidelines. As per the Ethical Guidelines for Clinical Studies, participation in this study will be preceded by the informed consent process. Considering the difficulty in obtaining assent, even implicitly, from the fetus, we believe that the parental permission for the fetus to participate.

13. ADDITIONAL NOTES RELATED TO THE ACT ON THE PROTECTION OF PERSONAL INFORMATION RELATED BY INDEPENDENT ADMINISTRATIVE INSTITUTIONS

1. Data Collection

Study data will be de-identified before being stored in electronic format. De-identified or anonymous data will be analyzed at Mie University. Joint researchers will examine and discuss the analyzed results.

2. Data Management

The results of analyses of the collected test data will be securely stored at the Secretariat located in Mie University.

3. Storage of Electronic Media

The results of analyses will be filed in electronic media, which will be kept securely in a locked room of Mie University. The Secretariat staff member, Dr. Takashi Umekawa, assumes the responsibility for data storage. In addition to the corresponding researcher, appointed members of the Secretariat staff will be granted access to the study data.

4. Method and Timing of Data De-identification

Registration numbers will be used to de-identify the study data at individual study sites. Each study site must ensure that the data they transfer to the Secretariat contains no explicit personal identifiers.

5. Notification of Analytical Results

Parents who participate in this study will not be informed of the results of this study.

14. REFERENCE

- 1 Minakami H, Maeda T, Fujii T, Hamada H, Iitsuka Y, Itakura A *et al.*
2 Guidelines for obstetrical practice in Japan: Japan Society of Obstetrics and Gynecology
3 (JSOG) and Japan Association of Obstetricians and Gynecologists (JAOG) 2014 edition. *J*
4 *Obstet Gynaecol Res* 2014; **40**: 1469–1499.
- 5 Kusuda S, Fujimura M, Sakuma I, Aotani H, Kabe K, Itani Y *et al.*
6 Morbidity and mortality of infants with very low birth weight in Japan: center variation.
7 *Pediatrics* 2006; **118**: e1130–e1138.
- 8 American College of Obstetricians and Gynecologists. ACOG Practice
9 bulletin no. 134: fetal growth restriction. *Obstet Gynecol* 2013; **121**: 1122–1133.
- 10 Hui L, Challis D. Diagnosis and management of fetal growth restriction: the
11 role of fetal therapy. *Best Pract Res Clin Obstet Gynaecol* 2008; **22**: 139–158.
- 12 Coppage KH, Sun X, Baker RS, Clark KE. Expression of phosphodiesterase
13 5 in maternal and fetal sheep. *Am J Obstet Gynecol* 2005; **193**: 1005–1010.
- 14 Rotella DP. Phosphodiesterase 5 inhibitors: current status and potential
15 applications. *Nat Rev Drug Discov* 2002; **1**: 674–682.
- 16 Wareing M, Myers JE, O’Hara M, Kenny LC, Warren AY, Taggart MJ *et al.*
17 Effects of a phosphodiesterase-5 (PDE5) inhibitor on endothelium-dependent relaxation of
18 myometrial small arteries. *Am J Obstet Gynecol* 2004; **190**: 1283–1290.
- 19 Wareing M, Myers JE, O’Hara M, Baker PN. Sildenafil citrate (Viagra)
20 enhances vasodilatation in fetal growth restriction. *J Clin Endocrinol Metab* 2005; **90**: 2550–
21 2555.
- 22 Herraiz S, Pellicer B, Serra V, Cauli O, Cortijo J, Felipe V *et al.* Sildenafil
23 citrate improves perinatal outcome in fetuses from pre-eclamptic rats. *BJOG Int J Obstet*
24 *Gynaecol* 2012; **119**: 1394–1402.
- 25 Ramesar SV, Mackraj I, Gathiram P, Moodley J. Sildenafil citrate improves
26 fetal outcomes in pregnant, L-NAME treated, Sprague-Dawley rats. *Eur J Obstet Gynecol*
27 *Reprod Biol* 2010; **149**: 22–26.
- 28 Baijnath S, Soobryan N, Mackraj I, Gathiram P, Moodley J. The
29 optimization of a chronic nitric oxide synthase (NOS) inhibition model of pre-eclampsia by
30 evaluating physiological changes. *Eur J Obstet Gynecol Reprod Biol* 2014; **182**: 71–75.
- 31 Nassar AH, Masrouha KZ, Itani H, Nader KA, Usta IM. Effects of sildenafil
32 in N ω -nitro-L-arginine methyl ester-induced intrauterine growth restriction in a rat model. *Am J*
33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

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5
6 *Perinatol* 2012; **29**: 429–434.

7 13 Cross JC, Hemberger M, Lu Y, Nozaki T, Whiteley K, Masutani M *et al.*
8 Trophoblast functions, angiogenesis and remodeling of the maternal vasculature in the placenta.
9 *Mol Cell Endocrinol* 2002; **187**: 207–212.

10 14 Watson ED, Cross JC. Development of structures and transport functions in
11 the mouse placenta. *Physiol Bethesda Md* 2005; **20**: 180–193.

12 15 von Dadelszen P, Dwinnell S, Magee LA, Carleton BC, Gruslin A, Lee B *et*
13 *al.* Sildenafil citrate therapy for severe early-onset intrauterine growth restriction. *BJOG Int J*
14 *Obstet Gynaecol* 2011; **118**: 624–628.

15 16 Sharp A, Comforth C, Jackson R, Turner M, Kenny L, Baker P *et al.*
16 OC01.05: STRIDER UK: a randomised controlled trial of sildenafil therapy in dismal prognosis
17 early-onset intrauterine growth restriction. *Ultrasound Obstet Gynecol* 2017; **50**: 3–3.

18 17 Sahni S, Palkar AV, Rochelson BL, Kępa W, Talwar A. Pregnancy and
19 pulmonary arterial hypertension: A clinical conundrum. *Pregnancy Hypertens Int J Womens*
20 *Cardiovasc Health* 2015; **5**: 157–164.

21 18 Wilkins MR, Wharton J, Grimminger F, Ghofrani HA. Phosphodiesterase
22 inhibitors for the treatment of pulmonary hypertension. *Eur Respir J* 2008; **32**: 198–209.

23 19 Fogue ST, Patterson BE, Bedding AW, Payne CD, Phillips DL, Wrishko
24 RE *et al.* Tadalafil pharmacokinetics in healthy subjects. *Br J Clin Pharmacol* 2006; **61**: 280–
25 288.

26 20 Yoshikawa K, Umekawa T, Maki S, Kubo M, Nii M, Tanaka K *et al.*
27 Tadalafil Improves L-NG-Nitroarginine Methyl Ester-Induced Preeclampsia With Fetal Growth
28 Restriction-Like Symptoms in Pregnant Mice. *Am J Hypertens In press.*

29 21 Kubo M, Umekawa T, Maekawa Y, Tanaka H, Nii M, Murabayashi N *et al.*
30 Retrospective study of tadalafil for fetal growth restriction: Impact on maternal and perinatal
31 outcomes. *J Obstet Gynaecol Res* 2017; **43**: 291–297.

32 22 Kubo M, Tanaka H, Maki S, Nii M, Murabayashi N, Osato K *et al.* Safety
33 and dose-finding trial of tadalafil administered for fetal growth restriction: A phase-1 clinical
34 study. *J Obstet Gynaecol Res* 2017; **43**: 1159–1168.

35 23 Cunningham F, Leveno K, Bloom S, Spong CY, Dashe J. *Williams*
36 *Obstetrics, 24e.* McGraw-Hill, 2014.

37 24 Ganzevoort W, Alfirevic Z, von Dadelszen P, Kenny L, Papageorgiou A,
38 van Wassenaer-Leemhuis A *et al.* STRIDER: Sildenafil Therapy In Dismal prognosis
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6 Early-onset intrauterine growth Restriction—a protocol for a systematic review with individual
7 participant data and aggregate data meta-analysis and trial sequential analysis. *Syst Rev* 2014; **3**:
8 23.

9
10
11 25 Parer JT, Ikeda T. A framework for standardized management of intrapartum
12 fetal heart rate patterns. *Am J Obstet Gynecol* 2007; **197**: 26–e1.

13
14 26 Shinichi Nishiuma, Kimiko Arakawa, Masanori Taketsuna, Nobuyuki
15 Kobayashi. Safety and effectiveness of tadalafil in patients with erectile dysfunction based on
16 post marketing surveillance study. *Jpn J Impot Res* 2012; **27**: 15–26.

17
18
19 27 Shinozuka N. Fetal biometry and fetal weight estimation: JSUM
20 standardization. *Ultrasound Rev Obstet Gynecol* 2002; **2**: 156–161.

21
22 28 Berkley E, Chauhan SP, Abuhamad A, Committee S for M-FMP. Doppler
23 assessment of the fetus with intrauterine growth restriction. *Am J Obstet Gynecol* 2012; **206**:
24 300–308.

25
26
27 29 ADCIRCA, INN - Tadalafil - WC500032789.pdf.
28 [http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/huma](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/001021/WC500032789.pdf)
29 [n/001021/WC500032789.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/001021/WC500032789.pdf) (accessed 19 Nov2017).
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CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4-6
	2b	Specific objectives or hypotheses	6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6-8
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	N/A
Participants	4a	Eligibility criteria for participants	6-7
	4b	Settings and locations where the data were collected	8 and 14
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6-8 and 11
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	8-11
	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample size	7a	How sample size was determined	13
	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	8
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	8
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	8
	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	8
Implementation	11	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	N/A
Blinding	a		

	11	If relevant, description of the similarity of interventions	8
	b		
Statistical methods	12	Statistical methods used to compare groups for primary and secondary outcomes	13
	12	Methods for additional analyses, such as subgroup analyses and adjusted analyses	N/A
Results			
Participant flow (a diagram is strongly recommended)	13	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	N/A
	13	For each group, losses and exclusions after randomisation, together with reasons	N/A
Recruitment	14	Dates defining the periods of recruitment and follow-up	N/A
	14	Why the trial ended or was stopped	N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	N/A
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	N/A
Outcomes and estimation	17	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	N/A
	17	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	N/A
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	N/A
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	N/A
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	14-15
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	14-15
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	14-15
Other information			
Registration	23	Registration number and name of trial registry	13
Protocol	24	Where the full trial protocol can be accessed, if available	N/A
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	15

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

BMJ Open

TADAFER II: Tadalafil treatment for fetal growth restriction - a study protocol for a multicenter randomized controlled phase II trial.

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Keywords:	Fetal growth restriction, Phosphodiesterase 5 inhibitor, Tadalafil, Phase II trial, Study protocol

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Manuscripts

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5 **1 Title: TADAFER II: Tadalafil treatment for fetal growth restriction - a study protocol for**
6 **2 a multicenter randomized controlled phase II trial.**
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10 Running head: Tadalafil for fetal growth restriction
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1 **Title: TADAFER II: Tadalafil treatment for fetal growth restriction - a study protocol for**
2 **a multicenter randomized controlled phase II trial.**

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5 **ABSTRACT**

6 **Introduction:** There is no proven therapy to reverse or ameliorate fetal growth restriction
7 (FGR). Sildenafil, a selective phosphodiesterase 5 (PDE5) inhibitor, has been reported to
8 potentially play a therapeutic role in FGR, but this has not been established. Tadalafil is also a
9 selective PDE5 inhibitor. We have demonstrated the efficacy of tadalafil against FGR along with
10 short-term outcomes and the feasibility of tadalafil treatment. Based on the hypothesis that
11 tadalafil will safely increase the likelihood of increased fetal growth in FGR, we designed this
12 phase II study to prospectively evaluate the efficacy and safety of tadalafil against FGR.

13 **Methods and analysis:** This study is a multicenter randomized controlled phase II trial. A total
14 of 140 fetuses with FGR will be enrolled from medical centers in Japan. Fetuses will be
15 randomized to receive either the conventional management for FGR or a once-daily treatment
16 with 20 mg of tadalafil along with the conventional management until delivery. The primary
17 endpoint is the fetal growth velocity from the first day of the protocol-defined treatment to birth
18 (g/day).

19 To minimize bias in terms of fetal baseline conditions and timing of delivery, a fetal indication
20 for delivery was established in this study. The investigator will evaluate fetal baseline conditions
21 at enrollment and will decide the timing of delivery based on this fetal indication. Infants will be
22 followed-up for development until 1.5 years of age.

23 **Ethics and dissemination:** This study was approved by the Institutional Review Board of Mie
24 University Hospital and each participating institution. Our findings will be widely disseminated
25 through peer-reviewed publications.

26 **Trial registration:** UMIN000023778.

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1 **Strengths and limitations of this study**

- 2 • This is a multicenter randomized controlled phase II trial to prospectively evaluate the
3 efficacy and safety of tadalafil treatment in fetuses with fetal growth restriction (FGR), for
4 which there is no proven therapy.
- 5 • Participants include major medical centers providing treatment for fetuses with FGR
6 according to the guidelines for obstetrical practice in Japan.
- 7 • To minimize bias in terms of fetal baseline conditions and timing of delivery, a fetal
8 indication for delivery was established in this study on the basis of the results from a
9 multicenter survey in Japan.
- 10 • The possible limitation is related to open-label trial features, in which enrolled participants
11 receive either the conventional management for FGR according to the guidelines for
12 obstetrical practice in Japan or a once-daily treatment with 20 mg of tadalafil in addition to
13 the conventional management.
- 14 • It is possible that SGA was included among the cases of FGR without Doppler
15 abnormalities in this study.

1 INTRODUCTION

2 Fetal growth restriction (FGR) is a common complication of pregnancy that is
3 associated with a variety of adverse perinatal outcomes. Although the main indication
4 when treating FGR is to consider the appropriate termination of the pregnancy, fetal
5 prematurity depending on gestational age is a serious problem, as shown by the
6 multicenter survey of very low birth weight (VLBW) infants in Japan conducted by
7 Kusuda and Ikeda et al [1]. There is also no proven fetal therapy to reverse or
8 ameliorate established FGR [2]. To prevent FGR, nutritional and dietary
9 supplementation, bed rest, and aspirin therapy have been investigated, but there is
10 insufficient evidence for the routine indication of any of these treatments [3].

11 Fetal growth is promoted by an adequate increase of utero-placental perfusion.
12 Vasodilation in the uteroplacental unit is probably due to the production and local
13 release of nitric oxide (NO), which stimulates cyclic guanosine monophosphate (cGMP)
14 production [4]. cGMP is inactivated mainly by phosphodiesterase (PDE), and PDE5
15 exists mainly in vascular smooth muscle cells.

16 It is now expected that PDE inhibitors could become therapeutic agents for FGR
17 in light of the inhibitors' artery dilation function, as confirmed in studies of erectile
18 dysfunction and pulmonary hypertension [5]. Sildenafil, a selective PDE5 inhibitor, has
19 been shown to improve endothelial function in myometrial small arteries removed from
20 women with pre-eclampsia and FGR [6, 7]. Although some reports have described an
21 effect of sildenafil on maternal hypertension, the effectiveness of sildenafil for rats with
22 FGR induced by L-NG-nitroarginine methyl ester (L-NAME) was not shown except in
23 Baijnath et al. [8-11]. They reported that sildenafil improved the fetal growth of FGR
24 induced by L-NAME [10]. Their study showed the improvement of growth from 4 days
25 postcoitum (d.p.c.) to 8 d.p.c, but not from 8 d.p.c to 14 d.p.c. In mouse placenta,
26 chorioallantoic attachment occurs at 8 d.p.c, and the mature circulatory pattern of
27 maternal blood through the placenta is established by 10 d.p.c [12, 13]. In a clinical
28 study, it was reported that sildenafil was associated with increased fetal abdominal
29 circumference (AC) growth velocity in severe early-onset FGR, but the authors did not
30 describe the fetal growth velocity or birth weight [14]. The STRIDER UK group
31 recently reported obtaining no evidence of a beneficial effect of sildenafil on survival or
32 short-term neonatal outcomes [15].

33 Tadalafil is another selective PDE5 inhibitor with a longer half-life and a more
34 rapid onset of action compared to sildenafil [5]. There are several reports showing the
35 safety of tadalafil treatment for pregnant women [16-18]. Regarding the plasma
36 concentration and the bioavailability of the drug, tadalafil is less susceptible to the

1 intake of a high-fat meal and less influenced by sex compared to sildenafil [19, 20]. Our
2 animal experiments demonstrated that tadalafil treatment dilates the maternal blood
3 sinuses in the placenta, which leads to increased placental growth factor (PIGF)
4 production and contributes to the facilitation of fetal growth [21]. In those animal
5 experiments, we can safely presume that tadalafil treatment contributed to the
6 facilitation of fetal growth via mechanisms associated with NO signaling, because the
7 tadalafil treatment was initiated after blood spaces in the placenta were narrowed by L-
8 NAME treatment, and an elevated urinary excretion of cGMP was observed.

9 We also retrospectively analyzed the cases of 11 Japanese singleton pregnant
10 women showing FGR who received tadalafil along with conventional management for
11 FGR at Mie University Hospital from July 2015 to February 2016 (the tadalafil group)
12 [22]. The women were matched for maternal age, parity, and gestational age (GA), and
13 we also estimated fetal weight at enrollment among 14 singleton pregnant women who
14 had received only the conventional management for FGR in 2014 (the conventional
15 management group). The conventional management for FGR was performed according
16 to the guidelines for obstetric practice in Japan [23]. This retrospective study showed
17 that both fetal growth velocity from enrollment to birth and birth weight were
18 significantly higher in the tadalafil group than in the conventional management group.
19 Moreover, the prevalence of respiratory distress syndrome (RDS) was significantly
20 lower in the tadalafil group compared to the conventional management group.

21 As the next step, a phase I trial was conducted to confirm the safety of tadalafil
22 administration for FGR [24]. No severe adverse event was seen following the initiation
23 of a daily tadalafil dose of 10 mg, 20 mg, or 40 mg except for one intrauterine fetal
24 death case. That case was immediately reviewed by the safety evaluation committee,
25 which concluded that the intrauterine fetal death was due to velamentous insertion of
26 the umbilical cord [25]. We concluded that tadalafil treatment was feasible in pregnant
27 women showing FGR [24].

28 Based on the above data, we hypothesized that tadalafil therapy can safely
29 increase the likelihood of increased fetal growth in fetuses with FGR, and we designed
30 the present multicenter randomized controlled phase II study to further examine the
31 efficacy and safety of fetal therapy with tadalafil. This study, funded by the Japan
32 Agency for Medical Research and Development (AMED), will prospectively evaluate
33 the safety and efficacy of tadalafil against FGR with the participation of major medical
34 centers providing treatment for fetuses with FGR according to the guidelines for
35 obstetrical practice in Japan.

36

METHODS

Study design

This study is a multicenter randomized controlled phase II trial.

Study period

The planned study period is from the date of ethics approval to February 2021. The Patient Registration Period will last until December 2018 starting from the date of ethics approval. The data collection and follow-up will be performed until 1.5 years after birth of the children registered under this study. Data collected by the end of the Neonatal Evaluation Period will be subjected to statistical analysis.

Patient selection

Inclusion criteria are as follows: (1) Pregnant women ≥ 20 years; (2) Estimated fetal weight (EFW) should be less than 1.5 standard deviations of the mean EFW for GA; (3) GA should be between 20 + 0 and 33 + 6 weeks; (4) The expected date of confinement is determined using the criteria of the guidelines for obstetrical practice in Japan (2014); (5) Only singleton pregnant patients should be selected; (6) Signed written informed consent should be obtained from the patients.

Exclusion criteria are as follows: (1) A result from the antepartum fetal tests, done at enrollment, which indicates that delivery should be attempted*; (2) A history of allergy to tadalafil; (3) Concurrent medications that interact adversely with tadalafil; (4) Contraindication of tadalafil treatment due to renal disease; (5) Contraindication of tadalafil treatment due to liver disease; (6) Contraindication of tadalafil treatment due to uncontrolled arrhythmia, hypertension (BP > 170/100 mmHg), and hypotension (BP < 80/40 mmHg); (7) Fetus with suspected chromosomal disorder and/or multiple congenital anomalies; (8) Contraindication of tadalafil treatment due to retinitis pigmentosa, coagulation defect, active gastric and/or intestinal ulcer, or venous obstructive disease; and (9) The investigators' decision that the entry is inappropriate**.

* To minimize bias in terms of fetal baseline condition at enrollment, a fetal indication for delivery was established on the basis of the results from the multicenter survey of VLBW infants in Japan using a network database, in which the 82 level III perinatal centers were registered. The survey data included infant survival rate in the NICU, categorized by birth weight and gestational week at birth (Figure 1) [1]. The infant survival rate data acquired from the survey were preprocessed with the moving average method and divided into three groups. The first group was defined as "Zone 1" where the infant survival rate in the NICU was less than 60%. The second group was defined as "Zone 2" where the infant survival rate in the NICU ranged from 60 to 95%. The third group was defined as "Zone 3" where the infant

1 survival rate in the NICU was 95% or higher. All patients in our study will undergo antepartum
 2 fetal tests consisting of the evaluation of fetal well-being by ultrasonography, including Doppler
 3 imaging of umbilical arterial blood flow, non-stress test, contraction stress test, and biophysical
 4 profile scoring depending on GA, to evaluate possible pregnancy termination by the investigator
 5 at enrollment (Table 1) [23, 25, 26].

6 **Table 1. A fetal indication for delivery in the TADAFER II study** [23, 25, 26]

Infant survival rate in the NICU (See Figure 1)	
Zone 1	Decide timing of delivery depending on available therapeutic measures at the NICU in each institute.
Zone 2	Consider delivery if at least one of three findings is made, but place a high priority on the determination by the investigators. <ol style="list-style-type: none"> 1. Reversed umbilical artery blood flow during diastole 2. Score less than 4 on the fetal biophysical profile score 3. Fetal heart rate patterns in the orange or red category for more than 30 minutes [26]
Zone 3	Consider delivery if at least one of the following five findings is made, but place a high priority on the determination by the investigators. <ol style="list-style-type: none"> 1. Reversed or absent umbilical artery blood flow during diastole 2. Score less than 4 on the fetal biophysical profile score (Score less than 6 on the fetal biophysical profile score if oligohydramnios is present) 3. Fetal heart rate patterns in the orange or red category for more than 30 minutes [26] 4. Positive contraction stress test 5. Impaired fetal head circumference growth for more than 2 weeks

7 ****Regarding exclusion criterion No.9 (The investigator decides that entry is inappropriate), this**
 8 **study excludes mothers with mental or psychiatric problems, since poor judgment capabilities**
 9 **that are often associated with such conditions may not be compatible with Inclusion Criterion**
 10 **No. 6.**

12 **Registration**

13 The study protocol defines all the procedures and schedules that the investigator must abide
 14 by to complete this clinical study, including patient selection and registration, fetal treatment of
 15 FGR, and follow-up (Figure 2). Patients that satisfy all inclusion criteria and do not meet any of
 16 the exclusion criteria will be eligible for inclusion in the study. Individual study sites will be
 17 responsible for guiding potential participants through the informed consent process, including
 18 patients who have been referred to them for treatment purposes. The investigator will enter an
 19 eligible patient's information into the Eligibility Confirmation Form on the website of this
 20 clinical trial (the Clinical Trial Data Management System: Japanese-only website). The data
 21 management system will check the contents of the form before registering the patient. For
 22 patients who meet all inclusion criteria without violating any of the exclusion criteria listed
 23 above, the data management system will register and allocate them to the two arms in an

1 allocation ratio of 1:1, one group receiving the conventional management of FGR according to
2 the guidelines for obstetrical practice in Japan [23] and the other receiving once-daily treatment
3 with 20 mg tadalafil added to conventional management after adjustment for study sites and GA
4 (< 28 or ≥ 28 weeks of gestation). The investigators are blinded to the allocation algorithm.
5 Enrolled participants will receive fetal therapy within 7 days of registration. The investigator
6 will enter the patients' data into the Case Report Form on the website of this clinical trial (the
7 Clinical Trial Data Management System).

9 **Fetal Treatment Protocol**

10 The investigator will provide the fetal therapy as described below.

11 *Arm A:* The conventional management of FGR according to the guidelines for obstetrical
12 practice in Japan will be followed [23]. Briefly, the conventional management of FGR consists
13 of evaluation of fetal well-being on ultrasonography, including Doppler imaging of umbilical
14 arterial blood flow, non-stress test, contraction stress test, and biophysical profile scoring
15 depending on GA to evaluate possible pregnancy termination.

16 *Arm B:* Patients will be provided a once-daily treatment with 20 mg tadalafil added to the
17 conventional management until delivery.

18 The investigators are blinded to the allocation algorithm. Enrolled participants will receive fetal
19 therapy within 7 days of registration.

21 **Endpoints**

22 **(1) Primary endpoint**

23 The primary endpoint is fetal growth velocity from the first day of the protocol-defined
24 treatment to birth (g/day), and is calculated using the following formula:

$$\begin{aligned} & \text{Fetal growth velocity (g/day)} \\ & = \frac{\text{Birthweight} - \text{EFW at the first day of the treatment [g]}}{\text{Days of the treatment [days]}} \end{aligned}$$

25 **Rationale for the primary endpoint**

26 Our primary hypothesis is that tadalafil therapy will increase the likelihood of increased fetal
27 growth velocity in fetuses with FGR. Taking into account the results of our retrospective study
28 demonstrating that tadalafil treatment increased fetal growth velocity (g/day), which was the
29 primary outcome of the retrospective study, and decreased the incidence of RDS; an
30 improvement in fetal growth velocity from the first day of the protocol-defined treatment to
31 birth (g/day) is an important indicator of the therapeutic benefits for fetuses with FGR [22]. The
32 cases of fetal death were included in analysis of primary endpoint.

33 **(2) Secondary endpoints**

1) Completion rate of the treatment regimen.

Completion rate of the treatment regimen is defined as the percentage of enrolled patients who receive the protocol-defined treatment for more than 7 days.

2) Efficacy endpoints.

i) Estimated fetal weight (g).

Estimated fetal weight (EFW) is calculated using the following formula [27]:

$$\text{EFW (g)} = 1.07 \times (\text{biparietal diameter or BPD})^3 + 0.3 \\ \times (\text{abdominal circumference or AC})^2 \times (\text{femur length or FL})$$

ii) Fetal growth velocity in the two weeks after the protocol-defined treatment and in the two weeks after one week of the protocol-defined treatment (g/day).

Fetal growth velocity in the two weeks after the protocol-defined treatment (g/day) is calculated using the following formula:

$$\text{Fetal growth velocity in the two weeks after the protocol – defined treatment (g/day)} \\ = \frac{(\text{EFW two weeks after the treatment} - \text{EFW at the first day of the treatment [g]})}{14 \text{ [days]}}$$

, and fetal growth velocity in the two weeks after one week of the protocol-defined treatment (g/day) is calculated using the following formula:

$$\text{Fetal growth velocity in the two weeks after one week of the treatment (g/day)} \\ = \frac{(\text{EFW three weeks after the treatment} - \text{EFW one week after the treatment [g]})}{14 \text{ [days]}}$$

iii) Fetal growth rate in the two weeks after the protocol-defined treatment and from the first day of the protocol-defined treatment to birth (%/day).

Fetal growth rate in the two weeks after the protocol-defined treatment (%/day) is calculated using the following formula:

$$\text{Fetal growth rate in the two weeks after the protocol-defined treatment (%/day)} \\ = \frac{(\text{EFW two weeks after the treatment} - \text{EFW at the first day of the treatment [g]})}{\text{EFW at the first day of the treatment [g]} \times 100} \\ \times 100 \\ = \frac{\text{EFW two weeks after the treatment} - \text{EFW at the first day of the treatment [g]}}{14 \text{ [days]}}$$

, and fetal growth rate from the first day of the protocol-defined treatment to birth (%/day) is calculated using the following formula:

$$\text{Fetal growth rate from the first day of the protocol-defined treatment to birth (%/day)} \\ = \frac{(\text{Birthweight} - \text{EFW at the first day of the treatment [g]})}{\text{EFW at the first day of the treatment [g]} \times 100} \\ \times 100 \\ = \frac{\text{Birthweight} - \text{EFW at the first day of the treatment [g]}}{\text{Days of the treatment [days]}}$$

iv) Fetal head circumference (cm).

1 The fetal head circumference was measured at the plane of the third ventricle with the thalamus
2 in the central portion and the cavum septi pellucidi visible in the anterior portion.

3 v) Doppler imaging of umbilical arterial blood flow.

4 Umbilical arterial blood flow was examined by Doppler ultrasound according to the Society for
5 Maternal-Fetal Medicine (SMFM) Clinical Guidelines [28].

6 vi) Deepest amniotic fluid pocket (cm).

7 The deepest amniotic fluid pocket was measured by transabdominal ultrasonography.

9 Fetal head circumference, deepest amniotic fluid pocket, and Doppler imaging of umbilical
10 arterial blood flow are evaluated according to the flow chart as shown below:

16 **Fetal head circumference, deepest amniotic fluid pocket, and Doppler imaging of umbilical**
17 **arterial blood flow evaluation flow chart.**

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5		
	Day of enrollment	1 week after the enrollment	2 weeks after the enrollment	3 weeks after the enrollment	4 weeks after the enrollment	Every two weeks before 36 weeks of GA after visit 5	Every one week at or after 37 weeks of GA
Fetal head circumference	•	•	•	•	•	•	•
Deepest amniotic fluid pocket	•	•	•	•	•	•	•
Doppler imaging of umbilical arterial blood flow	•	•	•	•	•	•	•

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19 vii) Prolongation of GA at birth (days).

20 Prolongation of GA at birth is defined as days from the first day of the protocol-defined
21 treatment to birth.

22 viii) Birth weight (g).

23 Birth weight is defined as the weight of the infant at birth.

24 ix) GA at birth.

25 GA at birth is defined as the gestational age at birth.

26 x) Apgar score.

1 The Apgar score consists of an evaluation of five factors: heart rate, respiratory effort, muscle
2 tone, responsiveness, and color at one minute and five minutes after birth.

3 xi) Umbilical artery pH and base excess values.

4 Umbilical artery pH and base excess is measured at delivery.

5 xii) Incidence rate of pre-eclampsia.

6 Incidence rate of pre-eclampsia is defined as the percentage of enrolled patients who develop
7 pre-eclampsia after the protocol-defined treatment.

8 xiii) Pediatric developmental assessment until 1.5 years of age.

9 Pediatric developmental assessment includes physiological and neurological developmental
10 assessment, and infant complications including cerebral palsy, epilepsy, and death. In the
11 neurodevelopment test in this study, the Kyoto Scale of Psychological Development 2001 was
12 used. Evaluation of neurodevelopment was performed by a pediatric neurologist.

13 3) Safety endpoints

14 i) Incidence rate of obstetric complications.

15 Incidence rate of obstetric complications including hypertensive disorders of pregnancy (HDP)
16 is defined as the percentage of enrolled patients who develop obstetric complications after the
17 protocol-defined treatment.

18 ii) Perinatal mortality.

19 Perinatal mortality is defined to include stillbirths (occurring after 22 weeks of gestation) and
20 neonatal deaths (occurring up to 7 days after birth).

21 iii) Neonatal mortality.

22 Neonatal mortality is defined as neonatal deaths occurring up to 28 days after birth.

24 **Stopping Criteria**

25 The investigator must discontinue the protocol-defined treatment when certain events
26 prevent continuation of the protocol treatment. These events include the following:

27 1. The mother has withdrawn her consent for participation in this study.

28 2. Certain events prevent continuation of the protocol treatment, which include the following:

29 a) A serious adverse drug reaction to tadalafil has developed.

30 b) The investigator's decision to prioritize other management including termination of the
31 pregnancy instead of continuation of the protocol-defined treatment.

32 c) The investigator's decision that it is inappropriate to continue with the protocol treatment.

33 d) The mother's poor compliance or discontinuation of the protocol treatment.

35 **Criteria for Delivery**

36 In this study, to minimize bias in terms of the timing of delivery, a fetal indication for

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5 1 delivery is established on the basis of the results from the multicenter survey of VLBW infants
6 2 in Japan using a network database (Figure 1 and Table 1). After registration, all patients will
7 3 receive the conventional management of FGR according to the guidelines for obstetrical
8 4 practice in Japan regardless of the treatment arm [23]. Briefly, the conventional management of
9 5 FGR consists of the evaluation of fetal well-being on ultrasonography, Doppler imaging of
10 6 umbilical arterial blood flow, non-stress test, contraction stress test, and biophysical profile
11 7 scoring depending on GA, to evaluate possible pregnancy termination. The investigator will
12 8 evaluate the fetal condition and decide timing of delivery referring to Table 1. For other
13 9 complications such as preterm labor, rupture of the membranes, and hypertensive disorder of
14 10 pregnancy, the investigator will follow guidelines for obstetric practice in Japan [23]. The
15 11 investigator must provide a report that explains the reason for termination of the pregnancy on
16 12 the website of this clinical trial (the Clinical Trial Data Management System).
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24 **Monitoring Safety during the Fetal Therapy**

25 The investigator must pay close attention to the safety of not only the fetus but also the
26 16 mother. As shown in the study schedule, the protocol-defined assessments include evaluation of
27 17 maternal blood pressure and pulse rate, maternal blood and urine tests (blood
28 18 fibrinogen and anti-thrombin III levels, liver and renal function tests, serum electrolyte levels,
29 19 and qualitative urine protein excretion), and maternal serum PIGF and soluble FMS-like
30 20 tyrosine kinase receptor (sFLT-1) levels. Other assessments include adverse events assessed by
31 21 medical consultation and antepartum fetal tests consisting of ultrasonography including Doppler
32 22 imaging of blood flow (umbilical artery, middle cerebral artery, and uterine artery), non-stress
33 23 test, contraction stress test, and biophysical profile scoring depending on GA. The investigator
34 24 will enter patients' safety data into the Case Report Form on the website of this clinical trial (the
35 25 Clinical Trial Data Management System).
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43 **Safety Evaluation Committee**

44 28 The Safety Evaluation Committee is responsible for the overall safety of this clinical study.
45 29 To ensure the safety of the protocol-defined treatment, the Safety Evaluation Committee will
46 30 review the adverse events of tadalafil treatment. The safety committee will have blind access to
47 31 the data. If a seriously adverse event develops, the investigator will provide the Secretariat with
48 32 the necessary information within 24 hours of its onset, according to the predetermined
49 33 procedure. Then the Secretariat will forward the obtained information without delay to the
50 34 Safety Evaluation Committee for review. The Safety Evaluation Committee will notify the
51 35 investigator of the review results. If the adverse event is definitely or probably related to
52 36 tadalafil treatment, the Ethics Committee of Mie University Hospital or each institute will
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1 consider possible termination of this clinical study. Special attention must be paid to the
 2 reporting requirements stipulated in the Ethical Guidelines for Clinical Studies (Ministry of
 3 Health, Labor, and Welfare in Japan, 2017). Infants will be followed-up and evaluated for
 4 physiological and neurological development until 1.5 years of age.

5 **Sample size**

6 One hundred and forty fetuses and their mothers.

7 **Rationale for the Target Sample Size**

8 Table 2 shows the summary of the distribution of fetal growth velocity from enrollment to
 9 birth in our retrospective study [22]. We estimate that the distribution of fetal growth velocity of
 10 this prospective phase II trial will be similar to that of our retrospective study. When the results
 11 of our prospective study are analyzed by Wilcoxon Rank Sum Test and group comparisons, with
 12 α of 0.05, two sided, we will have 90% power to detect a difference if we randomize 62 women
 13 per group. Allowing for a 10% drop-out rate, the total sample size required is 140 women.

14 **Table 2. The distribution of fetal growth velocity from enrollment to birth in the**
 15 **retrospective study conducted at Mie University Hospital.**

Fetal growth velocity (g/day)	<5	≥5 to <10	≥10 to <15	≥15 to <20	≥20 to <25	≥25
The conventional management group (%)	5.3	10.5	21.1	47.3	15.8	0
The tadalafil group (%)	0	8.3	8.3	50.0	16.7	16.7

17 **Statistical analysis**

18 Analysis is done on all randomized fetuses who receive the protocol-defined treatment at
 19 least once, as the full analysis set. All randomized participants with outcome data available will
 20 be included in the analyses, which will be performed on an intention-to-treat basis, according to
 21 the treatment allocation at randomization. Analysis ~~per protocol set~~ full analysis set (i.e.,
 22 removing patients who do not meet the inclusion and exclusion criteria) is done as a secondary
 23 analysis population for sensitivity analysis. All outcome measures are presented as summaries
 24 of descriptive statistics (mean [SD] or median [minimum, maximum, and interquartile range] for
 25 continuous measures, and the numbers and proportions for ordinal and dichotomous measures).
 26 Descriptive statistics for the primary endpoint, i.e. fetal growth velocity from the first day of the
 27 protocol-defined treatment to birth (g/day), are analyzed for each treatment arm by the
 28 Wilcoxon Rank Sum Test and group comparisons. All analyses are performed according to a
 29 pre-specified statistical analysis plan. The Data Coordinating Center in Mie University Hospital
 30 supports the data management, statistical analysis, and reporting of the study.

31 **Ethics and dissemination**

1 This study was approved by the Institutional Review Board of Mie University Hospital on
2 August 25, 2016 (No.3041) prior to patient enrollment. The study protocol was also approved
3 by each institutional review board of all participating institutions. This study complies with the
4 Helsinki Declaration. Written informed consent will be obtained from all mothers before they
5 are recruited. This trial has been registered in the UMIN Clinical Trials Registry as
6 UMIN000023778 ([https://upload.umin.ac.jp/cgi-open-
7 bin/ctr/ctr_view.cgi?recptno=R000027132](https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno=R000027132)). Our findings will be widely disseminated through
8 conference presentations and peer-reviewed publications.

9 10 **Participating institutions**

11 Mie University, Showa University, Osaka University, Nagoya University, Mie Chuo Medical
12 Center, Municipal Yokkaichi hospital, Ise Red Cross Hospital, St. Marianna University,
13 Juntendo University, the Jikei University, Toho University, Yokohama City University Medical
14 Center, Kanagawa Children's Medical Center, Ehime University, Hamamatsu University School
15 of Medicine, Osaka Medical College, Niigata University, Showa University Northern Yokohama
16 Hospital, Showa University Koto Toyosu Hospital, Gifu University, University of the Ryukyus,
17 Shiga University, Shinshu University, Ehime Prefectural Central Hospital, Akita University,
18 Tokyo Metropolitan Bokutoh Hospital, Kyorin University, Tokyo Metropolitan Tama Medical
19 Center, Kuwana East Medical Center, Kanazawa University, Nagasaki Medical Center,
20 University of Toyama, Yamaguchi University, Toyota Memorial Hospital, Kainan Hospital,
21 Dokkyo Medical University, Saga Hospital, Kyoto Prefectural University, Toyama Central
22 Prefectural Hospital, Sapporo City General Hospital, Kagoshima University, Mie Prefectural
23 General Medical Center, Kyoto University, Sakakibara Heart Institute, and University of Fukui.

24 25 **Patient and Public Involvement**

26 Patients with FGR have helped to prioritize this research question through a James Lind
27 Alliance Priority Setting Partnership, which highlighted this as a priority topic. Patients were
28 not involved in the design of this study and in the recruitment to and conduct of this study. The
29 results of this study will be informed by homepage of Mie University Obstetrics and
30 Gynecology. For randomized controlled trials, there is the no burden of the intervention
31 assessed by patients themselves. Patients and or public were not involved in this trial.

32
33 The original protocol is available in *the supplementary file*.

34 35 **DISCUSSION**

36 This protocol has been already approved by the Institutional Review Board of Mie

1 University Hospital and 39 institutions in Japan. Fetuses with FGR will be enrolled from these
2 institutions. As the fetal growth velocity from the first day of the treatment to birth has been
3 defined as the primary endpoint and fetuses will be randomly assigned based on an open-label
4 design, timing of delivery should be made on the basis of similar criteria as much as possible.
5 This study is the first nation-wide intervention study in the field of obstetrics in Japan. We
6 selected an open-label study design with a strict fetal management algorithm on the basis of the
7 results from a multicenter Japanese survey instead of a placebo-controlled design because of
8 operational challenges including low acceptability by pregnant women in Japan. Each
9 participating medical center can provide treatment for fetuses with FGR by board certified
10 members of the Japan Society of Obstetrics and Gynecology, and the investigator will be able to
11 optimally decide timing of delivery according to the guidelines for obstetrical practice in Japan
12 [23]. To make more accurate decisions, a fetal indication for delivery is established in this study
13 on the basis of the results from the multicenter survey in Japan, in which 82 level III perinatal
14 centers, including 8 sites participating in this study, were registered (Table 1) [1]. The fetal
15 indication for delivery is divided into three groups depending on infant survival rate in the
16 NICU. As all patients will undergo antepartum fetal tests consisting of evaluation of fetal well-
17 being by ultrasonography, including Doppler imaging of umbilical arterial blood flow, non-
18 stress test, contraction stress test, and biophysical profile scoring depending on GA according to
19 the Japanese guidelines, the investigator can easily refer to this indication when deciding timing
20 of delivery. This indication will be used to evaluate fetal baseline condition at enrollment as
21 well. We believe that this approach could take advantage of strengths and minimize the possible
22 limitations related to open-label trial features.

23 We retrospectively compared the effect of tadalafil in patients with FGR and demonstrated
24 that both fetal growth velocity from enrollment to birth and birth weight were significantly
25 higher in the tadalafil group than in the conventional management group. The
26 required sample size of this prospective study was estimated based on the results of the
27 retrospective study that used the same primary outcome measure. Since patients with FGR were
28 enrolled in the retrospective study under similar criteria to those in this study, we think that it is
29 reasonable to use the results of the retrospective study for the estimation of the sample size.

30
31 **Contributors:** T.U., S.M., M.K., H.T., M.N., K.T., K.O., Y.K., M.E., T. Kimura, T. Kotani,
32 M.N., A.S., and T.I. helped in conception of the study. T.U. wrote the manuscript. S.T., Y.N.,
33 M.K., C.M., and M.N. provided the biostatistical study design. T.O. conducted statistical
34 analyses. T. I. is the principal Investigator of this trial and the grant holder. All authors have read
35 and approved the final manuscript.

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11 5 **Competing interests:** None declared.
12 6

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14 7 **Ethics approval:** The Institutional Review Board of Mie University Hospital on August 25,
15 8 2016 (No.3041).
16 9

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18 10 **Data sharing statement:** There is no requirement for data sharing in public research
19 11 expenditures of our funds, and we are not prepared for data sharing at present. In the future, if
20 12 the chief researcher receives requests, we will prepare for data sharing to the extent permitted by
21 13 the Japanese ethics guidelines.
22 14

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24
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30 20 Graduate School of Medicine) for his advice on the protocol of
31 21 pediatric developmental assessment.
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REFERENCES

- 1 Kusuda S, Fujimura M, Sakuma I, *et al.* Morbidity and mortality of infants with very low birth weight in Japan: center variation. *Pediatrics* 2006;**118**:e1130–e1138.
- 2 American College of Obstetricians and Gynecologists. ACOG Practice bulletin no. 134: fetal growth restriction. *Obstet Gynecol* 2013;**121**:1122–33.
- 3 Hui L, Challis D. Diagnosis and management of fetal growth restriction: the role of fetal therapy. *Best Pract Res Clin Obstet Gynaecol* 2008;**22**:139–58.
- 4 Coppage KH, Sun X, Baker RS, *et al.* Expression of phosphodiesterase 5 in maternal and fetal sheep. *Am J Obstet Gynecol* 2005;**193**:1005–10.
- 5 Rotella DP. Phosphodiesterase 5 inhibitors: current status and potential applications. *Nat Rev Drug Discov* 2002;**1**:674–82.
- 6 Wareing M, Myers JE, O'Hara M, *et al.* Effects of a phosphodiesterase-5 (PDE5) inhibitor on endothelium-dependent relaxation of myometrial small arteries. *Am J Obstet Gynecol* 2004;**190**:1283–90.
- 7 Wareing M, Myers JE, O'Hara M, *et al.* Sildenafil citrate (Viagra) enhances vasodilatation in fetal growth restriction. *J Clin Endocrinol Metab* 2005;**90**:2550–5.
- 8 Herraiz S, Pellicer B, Serra V, *et al.* Sildenafil citrate improves perinatal outcome in fetuses from pre-eclamptic rats. *BJOG Int J Obstet Gynaecol* 2012;**119**:1394–402.
- 9 Ramesar SV, Mackraj I, Gathiram P, *et al.* Sildenafil citrate improves fetal outcomes in pregnant, L-NAME treated, Sprague-Dawley rats. *Eur J Obstet Gynecol Reprod Biol* 2010;**149**:22–6.
- 10 Bajinath S, Soobryan N, Mackraj I, *et al.* The optimization of a chronic nitric oxide synthase (NOS) inhibition model of pre-eclampsia by evaluating physiological changes. *Eur J Obstet Gynecol Reprod Biol* 2014;**182**:71–5.
- 11 Nassar AH, Masrouha KZ, Itani H, *et al.* Effects of sildenafil in N^ω-nitro-L-arginine methyl ester-induced intrauterine growth restriction in a rat model. *Am J Perinatol* 2012;**29**:429–34.
- 12 Cross JC, Hemberger M, Lu Y, *et al.* Trophoblast functions, angiogenesis and remodeling of the maternal vasculature in the placenta. *Mol Cell Endocrinol* 2002;**187**:207–12.
- 13 Watson ED, Cross JC. Development of structures and transport functions in the mouse placenta. *Physiol Bethesda Md* 2005;**20**:180–93.
- 14 von Dadelszen P, Dwinnell S, Magee LA, *et al.* Sildenafil citrate therapy for severe early-onset intrauterine growth restriction. *BJOG Int J Obstet Gynaecol* 2011;**118**:624–8.
- 15 Sharp A, Comforth C, Jackson R, *et al.* OC01.05: STRIDER UK: a randomised controlled trial of sildenafil therapy in dismal prognosis early-onset intrauterine growth restriction. [abstract] *Ultrasound Obstet Gynecol* 2017;**50**:3.

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2
3
4
5 1 16 Sahni S, Palkar AV, Rochelson BL, *et al.* Pregnancy and pulmonary arterial
6 2 hypertension: A clinical conundrum. *Pregnancy Hypertens Int J Womens Cardiovasc Health*
7 3 2015;**5**:157–164.
8 4 17 Ladouceur M, Benoit L, Radojevic J, *et al.* Pregnancy outcomes in patients with
9 5 pulmonary arterial hypertension associated with congenital heart disease. *Heart Br Card Soc*
10 6 2017;**103**:287–92. 5t6tt
11 7 18 Daimon A, Kamiya CA, Iwanaga N, *et al.* Management of pulmonary vasodilator
12 8 therapy in three pregnancies with pulmonary arterial hypertension. *J Obstet Gynaecol Res*
13 9 2017;**43**:935–938.
14 10 19 Wilkins MR, Wharton J, Grimminger F, *et al.* Phosphodiesterase inhibitors for the
15 11 treatment of pulmonary hypertension. *Eur Respir J* 2008;**32**:198–209.
16 12 20 Forgue ST, Patterson BE, Bedding AW, *et al.* Tadalafil pharmacokinetics in healthy
17 13 subjects. *Br J Clin Pharmacol* 2006;**61**:280–288.
18 14 21 Yoshikawa K, Umekawa T, Maki S, *et al.* Tadalafil Improves L-NG-Nitroarginine
19 15 Methyl Ester-Induced Preeclampsia With Fetal Growth Restriction-Like Symptoms in Pregnant
20 16 Mice. *Am J Hypertens* 2017;**31**:89–96.
21 17 22 Kubo M, Umekawa T, Maekawa Y, *et al.* Retrospective study of tadalafil for fetal
22 18 growth restriction: Impact on maternal and perinatal outcomes. *J Obstet Gynaecol Res*
23 19 2017;**43**:291–297.
24 20 23 Minakami H, Maeda T, Fujii T, *et al.* Guidelines for obstetrical practice in Japan: Japan
25 21 Society of Obstetrics and Gynecology (JSOG) and Japan Association of Obstetricians and
26 22 Gynecologists (JAOG) 2014 edition. *J Obstet Gynaecol Res* 2014;**40**:1469–1499.
27 23 24 Kubo M, Tanaka H, Maki S, *et al.* Safety and dose-finding trial of tadalafil administered
28 24 for fetal growth restriction: A phase-I clinical study. *J Obstet Gynaecol Res* 2017;**43**:1159–
29 25 1168.
30 26 25 Cunningham F, Leveno K, Bloom S, *et al.* *Williams Obstetrics, 24e.* McGraw-hill 2014.
31 27 26 Parer JT, Ikeda T. A framework for standardized management of intrapartum fetal heart
32 28 rate patterns. *Am J Obstet Gynecol* 2007;**197**:26–e1.
33 29 27 Shinozuka N. Fetal biometry and fetal weight estimation: JSUM standardization.
34 30 *Ultrasound Rev Obstet Gynecol* 2002;**2**:156–161.
35 31 28 Berkley E, Chauhan SP, Abuhamad A, *et al.* Doppler assessment of the fetus with
36 32 intrauterine growth restriction. *Am J Obstet Gynecol* 2012;**206**:300–308.
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5 **FIGURE LEGENDS**

6 **Figure 1. Infant survival rate in the NICU categorized by birth weight and gestational**
7 **week at birth (%).**

8 This figure is established on the basis of the results from the multicenter survey of VLBW
9 infants in Japan using a network database. The survey data included infant survival rates in the
10 NICU, categorized by birth weight and gestational week at birth [1]. The infant survival rate
11 data acquired from the survey were preprocessed with the moving average method and divided
12 into three groups. The first group was defined as “Zone 1” where the infant survival rate in the
13 NICU was less than 60% (highlighted by a red background). The second group was defined as
14 “Zone 2” where the infant survival rate in the NICU ranged from 60 to 95% (highlighted by a
15 yellow background). The third group was defined as “Zone 3” where the infant survival rate in
16 the NICU was 95% or higher (highlighted by a blue background).
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24 **Figure 2. Summary of the study design.**
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Birth weight (g)	1401-1500						96	99	100	99	99	99	99
	1301-1400						94	97	99	99	99	100	99
	1201-1300						98	99	99	99	99	99	100
	1101-1200					96	96	99	100	99	99	99	100
	1001-1100				96	98	98	98	99	99	98	98	98
	901-1000				95	96	97	97	98	99	99	98	97
	801-900			89	91	95	96	96	97	97	98	100	100
	701-800		84	86	90	93	93	95	99	98	94	95	100
	601-700		78	86	90	93	94	93	96	100	100		
	501-600	59	69	80	90	87	93	94	92	87			
	401-500	49	64	71	80	77	80	86	100	71			
	301-400	41	52	51	56	68	67	73	71				
	201-300	18	10	31	33	40							
		22	23	24	25	26	27	28	29	30	31	32	33
Gestational week at birth													

Figure 1

Figure 1. Infant survival rate in the NICU categorized by birth weight and gestational week at birth (%).

173x177mm (300 x 300 DPI)

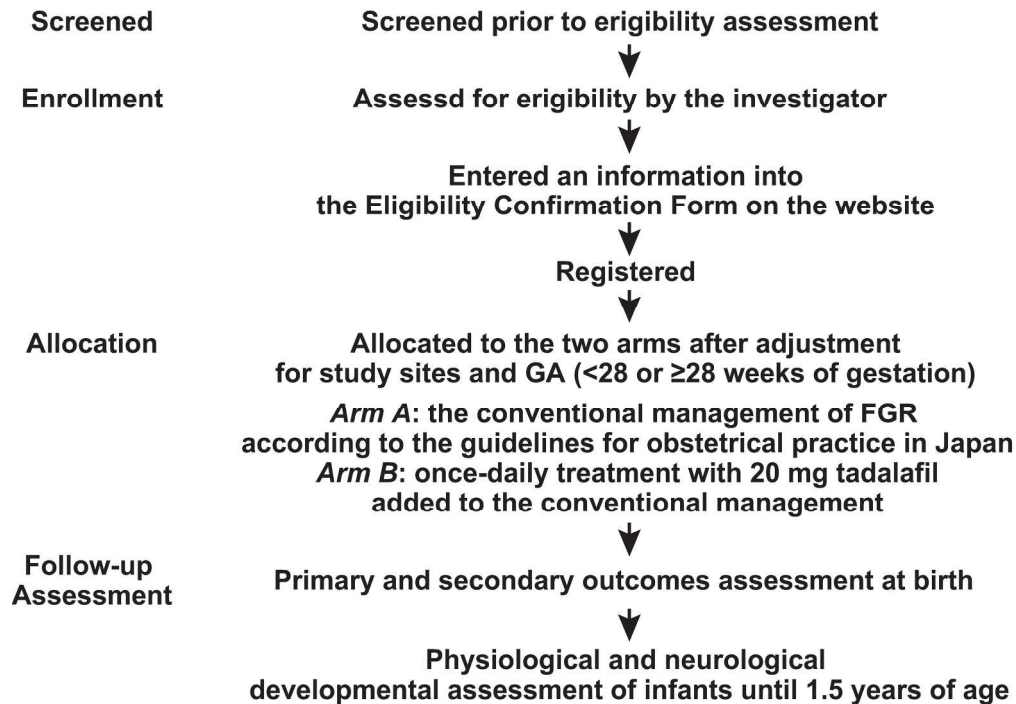


Figure 2

Figure 2. Summary of the study design.

212x193mm (300 x 300 DPI)

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Study Protocol

TADAFER II:

A multicenter phase II trial of the efficacy and safety of tadalafil in fetus with early-onset growth restriction.

Trial registration: UMIN Clinical Trials Registry UMIN000023778.

Version 1

Date 25-August-2016

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6 **Contents**

7 SYNOPSIS 3
8
9 1. VOLUNTARY PARTICIPATION AND WITHDRAWAL 5
10
11 2. BACKGROUD AND OBJECTIVES 5
12
13 3. RESEARCH ORGANIZATION AND PARTICIPATING INSTITUTIONS 8
14
15 4. STUDY SUBJECTS AND METHODS..... 10
16
17 5. TREATMENT AND COMPENSATION FOR STUDY-RELATED INJURY 19
18
19 6. STUDY PERIOD AND TARGET SAMPLE SIZE 20
20
21 7. OUTLINE OF THE STUDY PLAN..... 20
22
23 8. ANTICIPATED ADVERSE EVENTS..... 25
24
25 9. POTENTIAL BENEFITS AND RISKS 26
26
27 10. BURDEN OF COST..... 27
28
29 11. INTELLECTUAL PROPERTY RIGHTS 27
30
31 12. ETHICS 27
32
33 13. ADDITIONAL NOTES RELATED TO THE ACT ON THE PROTECTION OF
34 PERSONAL INFORMATION RELATED BY INDEPENDENT ADMINISTRATIVE
35 INSTITUTIONS..... 27
36
37 14. REFERENCE 29
38
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SYNOPSIS

1. Objectives

This multicenter randomized controlled phase II trial has been designed to prospectively evaluate the efficacy and safety of tadalafil treatment in fetus with fetal growth restriction (FGR).

2. Study eligibility

This study will include fetuses and their mothers who meet the following conditions:

- (1) Pregnant women ≥ 20 years.
- (2) Estimated fetal weight (EFW) less than 1.5 standard deviations of the mean EFW for gestational age.
- (3) Gestational age between 20 + 0 and 33 + 6 weeks.
- (4) The expected date of confinement is determined using the criteria of the guidelines for obstetrical practice in Japan (2014).¹
- (5) Singleton pregnancy.
- (6) Signed written informed consent.

3. Treatment

Fetuses with FGR will be randomized to receive either the conventional management of FGR according to the guidelines for obstetrical practice in Japan¹ or once-daily treatment with 20 mg tadalafil added to the conventional management until delivery.

4. Target sample size and duration of the study

Duration of the study: date of ethics approval to February 2021.

Target sample size: 140 singleton fetuses and their mothers.

5. Endpoints

- (1) Primary endpoint: fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day).
- (2) Secondary endpoints
 - 1) Completion rate of the treatment regimen
 - 2) Efficacy endpoints: estimated fetal weight (g), fetal growth velocity in the two weeks after the protocol-defined treatment (g/day), fetal growth velocity in the two weeks after one week of

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6 the protocol-defined treatment (g/day), fetal growth rate (%/day), fetal head circumference (cm),
7 Doppler imaging of umbilical arterial blood flow, deepest amniotic fluid pocket (cm),
8 prolongation of gestational age at birth (days), birth weight (g), gestational age (GA) at birth,
9 Apgar score, umbilical artery pH and base excess values, incidence rate of pre-eclampsia, and
10 pediatric developmental assessment until 1.5 years of age.
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14 3) Safety endpoints: incidence rate of obstetric complications, perinatal mortality, and neonatal
15 mortality.
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18 19 **6. Secretariats**

20
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1. VOLUNTARY PARTICIPATION AND WITHDRAWAL

Participation in this study is on a voluntary basis. Refusal to participate will incur no penalty or loss of benefits to which patients are otherwise entitled to. The subject may withdraw at any time without penalty.

2. BACKGROUD AND OBJECTIVES

Neonatal intensive care has improved over the past few decades, and morbidity among infants, including those who are premature, continues to decline. Premature infants with intrauterine growth restriction, however, still have high mortality and morbidity. The multicenter survey² of very low birth weight (VLBW) infants in Japan conducted by Kusuda and Ikeda *et al.* revealed that mortality in neonatal intensive care units (NICU), of small gestational age (SGA) infants born before 30 weeks gestation, was significantly higher than that of appropriate for gestational age (AGA) infants (unpublished data). To prevent fetal growth restriction (FGR), nutritional and dietary supplementation, bed rest, and aspirin therapy have been investigated, but there is insufficient evidence for the routine indication of any of these treatments.³ There is also no proven therapy to reverse or ameliorate established FGR.⁴

Increases in uteroplacental blood flow during pregnancy via angiogenesis and vasodilation contribute to adequate fetal growth. Vasodilation in the uteroplacental unit is considered to be due to the production and local release of nitric oxide (NO), which stimulates cyclic guanosine monophosphate (cGMP) production.⁵ cGMP is inactivated mainly by phosphodiesterases (PDE), and the predominant PDE isoform present in the vascular smooth muscle is PDE5. Because inhibitors of PDE5, which is a cGMP-specific PDE, exert their pharmacological action by dilating arteries and increasing blood flow, as proven in erectile dysfunction and pulmonary hypertension, recent studies have suggested a potential therapeutic role for PDE5 inhibitors in treating FGR.⁶ Sildenafil, a selective PDE5 inhibitor, has been shown to improve endothelial function in myometrial small arteries removed from women with pre-eclampsia and FGR.^{7,8} However, although sildenafil has been reported to affect maternal hypertension, it has not been shown to affect FGR in studies in FGR model rats induced by L-NG-nitroarginine methyl ester (L-NAME) treatment. In these studies, FGR was not affected by sildenafil except in one report, by Baijnath *et al.*^{9,10,11,12} Baijnath *et al.* demonstrated that L-NAME-induced FGR was improved by sildenafil treatment from 4 days postcoitum (d.p.c.) to

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6 8 d.p.c. but not from 8 d.p.c. to 14 d.p.c.¹¹ Chorioallantoic attachment occurs at 8 d.p.c., and the
7 mature circulatory pattern of maternal blood through the placenta is established by 10 d.p.c. in
8 mouse placenta.^{13,14} In considering the development of fetoplacental circulation in rodents, the
9 effect of sildenafil on fetal growth associated with placental blood flow via an NO-dependent
10 pathway was not manifested. In a clinical study, it was reported that sildenafil was associated
11 with increased fetal abdominal circumference (AC) growth velocity in severe early-onset FGR,
12 but the authors did not report on fetal growth velocity and birth weight.¹⁵ Recently, the
13 STRIDER UK group has found no evidence of a beneficial effect of sildenafil on survival or
14 short-term neonatal outcomes.¹⁶

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21 Tadalafil is also a selective PDE5 inhibitor and has a longer half-life and a more rapid
22 onset of action than sildenafil.⁶ Tadalafil has been used to treat pulmonary hypertension in
23 pregnant women and the Food and Drug Administration in the United States has rated tadalafil
24 as pregnancy category B.¹⁷ When taking sildenafil with a high-fat meal, the time to maximum
25 plasma concentration increases and the peak plasma concentration falls.¹⁸ In contrast, Forgue *et*
26 *al.* reported that food intake had a negligible effect on the bioavailability of tadalafil, and also
27 reported that there was no clinically meaningful effect of gender on tadalafil
28 pharmacokinetics.¹⁹ Our animal experiments demonstrated that tadalafil treatment dilates the
29 maternal blood sinuses in the placenta, which leads to increased placental growth factor (PIGF)
30 production, and contributes to facilitating fetal growth.²⁰ Because tadalafil treatment was started
31 after blood spaces in the placenta were narrowed by L-NAME treatment and elevated urinary
32 excretion of cGMP in these animal experiments, we can safely presume that tadalafil treatment
33 contributes to facilitating fetal growth in the context of the mechanisms associated with NO
34 signaling. In addition, we retrospectively analyzed 11 Japanese singleton pregnant women with
35 FGR who received tadalafil along with conventional management for FGR at Mie University
36 Hospital from July 2015 to February 2016 (tadalafil group).²¹ These women were matched for
37 maternal age, parity, gestational age (GA), and estimated fetal weight at enrollment with 14
38 singleton pregnant women who had received only the conventional management for FGR in
39 2014 (conventional management group). The conventional management for FGR was
40 performed according to the guidelines for obstetric practice in Japan.¹ This retrospective study
41 showed that both fetal growth velocity from enrollment to birth and birth weight were
42 significantly higher in the tadalafil group than in the conventional management group.
43 Moreover, the prevalence of respiratory distress syndrome (RDS) was significantly lower in the
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6 tadalafil group than in the conventional management group. After the retrospective study, we
7 conducted a phase I clinical trial to ensure the safety of tadalafil treatment for FGR.²² There
8 were no serious maternal adverse events for daily tadalafil doses of 10 mg, 20 mg, and 40 mg.
9 More patients who were administered 40 mg tadalafil daily experienced mild adverse events
10 than those administered 10 mg or 20 mg tadalafil daily. In regards to fetal adverse events,
11 intrauterine fetal death occurred in one case. In this case, the pregnant woman was prescribed 40
12 mg tadalafil daily and fetal growth had been progressing at a rate of 22 g/day. At 36 weeks
13 gestation, fetal movement suddenly ceased and a diagnosis of intrauterine fetal death was made.
14 Thereafter, the fetus was delivered vaginally, and velamentous insertion of the umbilical cord
15 was identified. Immediately, the safety evaluation committee investigated the incident's
16 relationship to tadalafil. This committee analyzed the case and concluded that the intrauterine
17 fetal death was due to velamentous insertion of the umbilical cord.²³ We concluded that tadalafil
18 treatment was feasible in pregnant women with FGR.²²

19 Based on the above, we have hypothesized that tadalafil therapy will safely increase the
20 likelihood of increased fetal growth in fetuses with FGR and have designed this multicenter
21 randomized controlled phase II study to establish evidence for fetal therapy with tadalafil. This
22 study, funded by the Japan Agency for Medical Research and Development (AMED), will
23 prospectively evaluate the safety and efficacy of tadalafil in FGR with the participation of major
24 medical centers providing treatment for fetuses with FGR according to the guidelines for
25 obstetrical practice in Japan. Fetuses will be randomized to receive either the conventional
26 management for FGR, according to the guidelines in Japan, or a once-daily treatment with 20
27 mg of tadalafil along with the conventional management, until delivery. Fetal growth velocity
28 from the first day of the protocol-defined treatment to birth (g/day) has been defined as the
29 primary endpoint in this study. To minimize bias in terms of fetal baseline condition and timing
30 of delivery, a fetal indication for delivery is established on the basis of the results from the
31 multicenter survey of VLBW infants in Japan using a network database, in which the 82 level
32 III perinatal centers were registered.² The investigator will evaluate fetal baseline conditions at
33 enrollment and will decide the timing of delivery based on this fetal indication. For other
34 complications such as preterm labor, rupture of the membranes, and hypertensive disorder of
35 pregnancy, the investigator will follow guidelines for obstetric practice in Japan.¹ The
36 investigator will enter the patients' data into the Case Report Form on the website of this
37 clinical trial (the Clinical Trial Data Management System). Infants will be followed up and
38 evaluated for physiological and neurological development until 1.5 years of age.

3. RESEARCH ORGANIZATION AND PARTICIPATING INSTITUTIONS

(1) Corresponding	Mie University	Tomoaki Ikeda (Principal Investigator)
(2) Collaborator	Showa University	Akihiko Sekizawa
	Osaka University	Tadashi Kimura
	Nagoya University	Tomomi Kotani
	Mie Chuo Medical Center	Yuka Maekawa
	Municipal Yokkaichi hospital	Kenji Nagao
	Ise Red Cross Hospital	Tomohisa Kihira
	St. Marianna University	Nao Suzuki
	Juntendo University	Satoru Takeda
	The Jikei University	Aikou Okamoto
	Toho University	Masahiko Nakata
	Yokohama City University Medical Center	Shigeru Aoki
	Kanagawa Children's Medical Center	Hiroshi Ishikawa
	Ehime University	Takashi Sugiyama
	Hamamatsu University School of Medicine	Naohiro Kanayama
	Osaka Medical College	Masahide Ohmichi
	Niigata University	Takayuki Enomoto
	Showa University Northern Yokohama Hospital	Kiyotake Ichizuka
	Showa University Koto Toyosu Hospital	Katsufumi Otsuki
	Gifu University	Kenichiro Morishige
	University of the Ryukyu	Yoichi Aoki
	Shiga University	Takashi Murakami
	Shinshu University	Tanri Shiozawa
	Ehime Prefectural Central Hospital	Hiroshi Ochi
	Akita University	Yukihiro Terada
	Tokyo Metropolitan Bokutoh Hospital	Hironobu Hyodo
	Kyorin University	Mitsutoshi Iwashita
	Tokyo Metropolitan Tama Medical Center	Akira Kohyama
	Kuwana East Medical Center	Yoshihito Sasaki
	Kanazawa University	Hiroshi Fujiwara
	Nagasaki Medical Center	Ichiro Yasuhi

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6	University of Toyama	Shigeru Saito
7	Yamaguchi University	Norihiro Sugino
8		
9	Toyota Memorial Hospital	Hidenori Oguchi
10		
11	Kainan Hospital	Tadashi Sumi
12	Dokkyo Medical University	Susumu Miyashita
13		
14	Saga Hospital	Makoto Nomiyama
15		
16	Kyoto Prefectural University	Jo Kitawaki
17		
18	Toyama Central Prefectural Hospital	Hiroshi Funamoto
19	Sapporo City General Hospital	Kazuhiko Okuyama
20		
21	Kagoshima University	Hiroaki Kobayashi
22		
23	Mie Prefectural General Medical Center	Hirohiko Tanaka
24	Kyoto University	Masaki Mandai
25		
26	Sakakibara Heart Institute	Shinji Katsuragi
27		
28	University of Fukui	Yoshio Yoshida

(3) Safety Evaluation Committee

The Safety Evaluation Committee is independent from research organization, and responsible for the overall safety of this clinical study. To ensure the safety of the protocol-defined treatment, the Safety Evaluation Committee will review adverse events of tadalafil. The Safety Evaluation Committee consists of Dr. Makoto Maeda (Board Certified Member of the Japan Society of Obstetrics and Gynecology) and Dr. Yoshiaki Miyake (Board Certified Member of the Japan Society of Obstetrics and Gynecology).

(4) Protocol Evaluation Committee

The Protocol Evaluation Committee is an organization of the execution of this study. All experimental protocols are evaluated and approved by the Protocol Evaluation Committee.

(5) Data Coordinating Center at the Clinical Research Support Center in Mie University Hospital

This center supports the data management, and statistical analysis and reporting of the study. This consists of Dr. Masakatsu Nishikawa (chairperson), Ms. Yuki Nishimura (data manager), and Dr. Toru Ogura (statistics).

(6) Secretariats

Dr. Takashi Umekawa, Dr. Shintaro Maki, and Dr. Michiko Kubo.

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4. STUDY SUBJECTS AND METHODS

(1) Study Sites and Subjects

1) Study Sites

This is a multicenter randomized controlled phase II trial, in which the Clinical Research Support Center in Mie University Hospital serves as the data center. Since this trial has been designed to prospectively evaluate the efficacy and safety of tadalafil treatment in FGR, fetuses will be randomized to receive either the conventional management for FGR, according to the guidelines in Japan¹ or a once-daily treatment with 20 mg of tadalafil along with the conventional management at the data center. Case registration requires the approval of the Ethics Committee. The following institutions will participate in this clinical trial:

Mie University, Showa University, Osaka University, Nagoya University, Mie Chuo Medical Center, Municipal Yokkaichi hospital, Ise Red Cross Hospital, St. Marianna University, Juntendo University, the Jikei University, Toho University, Yokohama City University Medical Center, Kanagawa Children's Medical Center, Ehime University, Hamamatsu University School of Medicine, Osaka Medical College, Niigata University, Showa University Northern Yokohama Hospital, Showa University Koto Toyosu Hospital, Gifu University, University of the Ryukyus, Shiga University, Shinshu University, Ehime Prefectural Central Hospital, Akita University, Tokyo Metropolitan Bokutoh Hospital, Kyorin University, Tokyo Metropolitan Tama Medical Center, Kuwana East Medical Center, Kanazawa University, Nagasaki Medical Center, University of Toyama, Yamaguchi University, Toyota Memorial Hospital, Kainan Hospital, Dokkyo Medical University, Saga Hospital, Kyoto Prefectural University, Toyama Central Prefectural Hospital, Sapporo City General Hospital, Kagoshima University, Mie Prefectural General Medical Center, Kyoto University, Sakakibara Heart Institute, and University of Fukui.

2) Subjects and Diagnostic Methods

All patients have to meet all inclusion criteria without violating any of the exclusion criteria listed below. All subjects will be followed-up until the end of the study.

Inclusion Criteria

(1) Pregnant women \geq 20 years.

- (2) EFW less than 1.5 standard deviations of the mean EFW for GA.
- (3) GA between 20 + 0 and 33 + 6 weeks.
- (4) The expected date of confinement is determined using the criteria of the guidelines for obstetrical practice in Japan (2014).
- (5) Singleton pregnancy.
- (6) Signed written informed consent.

Exclusion Criteria

- (1) A result from the antepartum fetal tests, done at enrollment, which indicates that delivery should be attempted.
- (2) A history of allergy to tadalafil.
- (3) Concurrent medications that interact adversely with tadalafil.
- (4) Contraindication of tadalafil treatment due to renal disease.
- (5) Contraindication of tadalafil treatment due to liver disease.
- (6) Contraindication of tadalafil treatment due to uncontrolled arrhythmia, hypertension (BP >170/100 mmHg), and hypotension (BP <80/40 mmHg).
- (7) Fetus with suspected chromosomal disorder and/or multiple congenital anomalies.
- (8) Contraindication of tadalafil treatment due to retinitis pigmentosa, coagulation defect, active gastric and/or intestinal ulcer, and venous obstructive disease.
- (9) The investigator decides to entry inappropriate.

Rationale for Eligibility Criteria

- When diagnosed as FGR, the mean EFW for GA but not the mean birthweight for GA should be used, and the estimated date of confinement using fetal measurements obtained during the early stage of pregnancy should be confirmed according to the guidelines for obstetrical practice in Japan (2014) in Inclusion Criteria Nos. 2 and 4.¹
- The lower age limit (20 weeks gestation) of Inclusion Criterion No. 3 is determined referring to the previous study protocol about the treatment for FGR.²⁴ The upper limit of <34 weeks gestation is based on infant survival rate in the NICU categorized by birth weight and gestational week at birth from the Japanese neonatal research network database (<http://nponrn.umin.jp/index.html> Japanese-only website), in which indicates that treatments are prioritized over elective delivery (Figure 1).

from 60 to 95%. The third group was defined as “Zone 3” where the infant survival rate in the NICU was 95% or higher. All patients in our study will undergo antepartum fetal tests consisting of the evaluation of fetal well-being by ultrasonography, including Doppler imaging of umbilical arterial blood flow, non-stress test, contraction stress test, and biophysical profile scoring depending on GA, to evaluate possible pregnancy termination by the investigator at enrollment (Table 1. Exclusion Criterion No. 1).

Table 1. A fetal indication for delivery in the TADAFER II study.^{1,23,25}

Infant survival rate in the NICU (See Figure 1)	
Zone 1	Decide timing of delivery depending on available therapeutic measures at NICU in each institute.
Zone 2	Consider delivery if at least one of three findings is made, but place give high priority on the determination by the investigators. <ol style="list-style-type: none"> 1. Reversed umbilical artery blood flow during diastole. 2. Score less than 4 on the fetal biophysical profile score. 3. Feat heart rate patterns in the orange or red category for more than 30 minutes.²⁵
Zone 3	Consider delivery if at least one of five findings is made, but place give high priority on the determination by the investigators. <ol style="list-style-type: none"> 1. Reversed or absent umbilical artery blood flow during diastole. 2. Score less than 4 on the fetal biophysical profile score. (Score less than 6 on the fetal biophysical profile score if oligohydramnios is present.) 3. Feat heart rate patterns in the orange or red category for more than 30 minutes.²⁵ 4. Positive contraction stress test. 5. Impaired fetal head circumference growth for more than 2 weeks.

- Patients who have contraindications for tadalafil treatment will be excluded (Exclusion Criteria from No.2 to No.7).
- Regarding exclusion criteria No.9, this study excludes mothers with mental or psychiatric problems, since poor judgment capabilities that are often associated with such conditions may not be compatible with Inclusion Criterion No. 6.

(2) Study Design

This study is a multicenter randomized controlled phase II trial.

(3) Methods

In this multicenter clinical study, each study site will obtain ethics approval of the protocol before its implementation.

Registration

This study protocol defines all the procedures and schedules that the investigator must abide by to complete this clinical study, including patient selection and registration, fetal treatment of FGR, and follow-up (Figure 2).

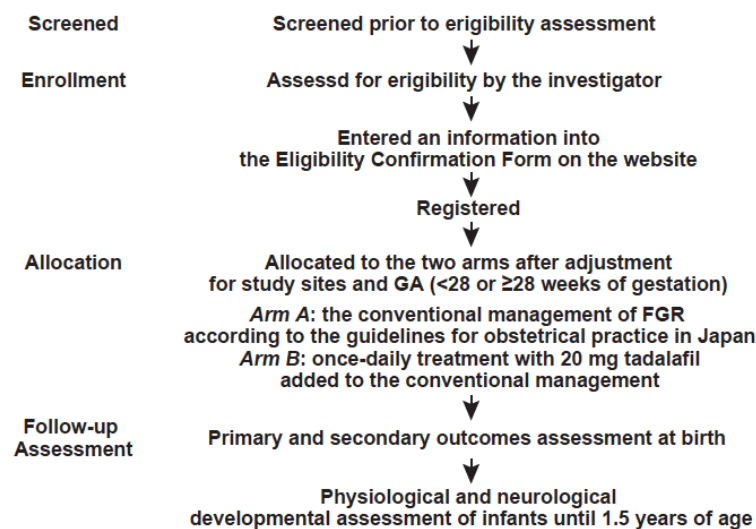


Figure 2. Summary of the study design.

The Clinical Research Support Center in Mie University Hospital will provide data center services including data management and patient registration. Patients that satisfy all inclusion criteria and do not meet any of the exclusion criteria will be eligible for inclusion in the study. Individual study sites will be responsible for guiding potential participants through the informed consent process, including patients who have been referred to them for treatment purposes. The investigator will enter an eligible patient's information into the Eligibility Confirmation Form on the website of this clinical trial (the Clinical Trial Data Management System: Japanese-only website). The data management system will check the contents of the form before registering the patient. For patients who meet all inclusion criteria without violating any of the exclusion criteria listed above, the data management system will register and allocate them to the two arms in an allocation ratio of 1:1, one group receiving the conventional management of FGR according to the guidelines for obstetrical practice in Japan,¹ and the other receiving once-daily treatment with 20 mg tadalafil added to conventional management after adjustment for study

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6 sites and GA (<28 or ≥28 weeks of gestation). The investigators are blinded to the allocation
7 algorithm. Enrolled participants will receive fetal therapy within 7 days of registration. The
8 investigator will enter the patients' data into the Case Report Form on the website of this
9 clinical trial (the Clinical Trial Data Management System).
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12 The corresponding researcher at Mie University will be responsible for the management of this
13 study (patient registration, data management, and coordination with the study-related
14 committees and the Clinical Research Support Center in Mie University Hospital). The
15 corresponding researcher will also be responsible for the research administration, scheduling,
16 documentation, and safety information management. The Safety Evaluation Committee will
17 assume responsibility for the safety of this study. The Clinical Research Support Center in Mie
18 University Hospital will provide technical support from the planning to the completion of this
19 clinical study. Its Data Management Department will manage the study data in cooperation with
20 the corresponding researcher and secretariats, and its Statistics Department will provide
21 statistical support to facilitate the efficacy evaluation. The Protocol Evaluation Committee is an
22 organization of the execution of this study. All experimental protocols are evaluated and
23 approved by the Protocol Evaluation Committee.
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34 **UMIN Clinical Trials Registry UMIN000023778.**

35 36 37 **Fetal Treatment Protocol**

38 The investigator will provide the fetal therapy as described below.

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40 *Arm A:* the conventional management of FGR according to the guidelines for obstetrical
41 practice in Japan.¹ Briefly, the conventional management of FGR consists of evaluation of fetal
42 well-being on ultrasonography, including Doppler imaging of umbilical arterial blood flow,
43 non-stress test, contraction stress test, and biophysical profile scoring depending on GA to
44 evaluate possible pregnancy termination.
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47 *Arm B:* once-daily treatment with 20 mg tadalafil added to the conventional management until
48 delivery.
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51 The investigators are blinded to the allocation algorithm. Enrolled participants will receive fetal
52 therapy within 7 days of registration.
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55 **Rationale for Dose Selection**

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57 Tadalafil was approved for treatment of erectile dysfunction (ED) in July 2007 in Japan.
58 Nishiuma S *et al.* reported the results from a post marketing surveillance study on tadalafil, with
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6 a primary goal of confirming the safety and effectiveness of tadalafil in Japanese patients with
7 ED in routine clinical practice. 86.7 % of the participants in the surveillance study were
8 prescribed 10mg or 20mg tadalafil daily.²⁶ We referred the results of adverse events in the
9 surveillance study and determined the dose of tadalafil in our retrospective study, in which three
10 pregnant women (27.3%) were prescribed 10 mg tadalafil daily and eight pregnant women
11 (72.7%) were prescribed 20 mg daily.²¹ In our phase I study, more patients who were
12 administered 40 mg tadalafil daily experienced adverse events than those administered 10 mg or
13 20 mg tadalafil daily, but we found that there were no serious maternal adverse events.²² Finally,
14 the minimum required sample size was estimated based on the results of our retrospective study.
15 Taken together, the tadalafil dosage (once-daily treatment with 20 mg) was set in this study.
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24 **Stopping Criteria**

25 The investigator must discontinue the protocol-defined treatment when certain events prevent
26 continuation of the protocol treatment. These events include the following:
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- 28 1. The mother has withdrawn her consent to study participation.
- 29 2. Certain events prevent continuation of the protocol treatment, which include the following:
 - 30 a) A serious adverse drug reaction to tadalafil has developed.
 - 31 b) The investigator's decision to prioritize other management including termination of the
 - 32 pregnancy instead of continuation of the protocol-defined treatment.
 - 33 c) The investigator's decision that it is inappropriate to continue with the protocol treatment.
 - 34 d) The mother's poor compliance or discontinuation of the protocol treatment.

35 Fetuses in whom the fetal therapy was prematurely discontinued as per the Stopping Criteria
36 will receive scheduled examinations and other assessments to the extent possible. If the mother
37 withdraws her consent to study participation, she and her fetus will be removed from the study.
38 If the fetal therapy is prematurely discontinued due to a serious adverse drug reaction to
39 tadalafil, scheduled subsequent examinations and other assessments should be continued to the
40 extent possible and the investigator should provide the patient experiencing an adverse event
41 with the most appropriate therapeutic measures available. If a registered mother or her fetus is
42 found to have been non-conformant to the eligibility criteria, poor compliance and dropping out
43 with the protocol treatment, the mother or fetus will be categorized as noncompliant.
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57 **Criteria for Delivery**

58 In this study, to minimize bias in terms of the timing of delivery, a fetal indication for delivery
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6 is established on the basis of the results from the multicenter survey of VLBW infants in Japan
7 using a network database (Figure 1 and Table 1). After registration, all patients will receive the
8 conventional management of FGR according to the guidelines for obstetrical practice in Japan
9 regardless of the treatment arm.¹ Briefly, the conventional management of FGR consists of the
10 evaluation of fetal well-being on ultrasonography, including Doppler imaging of umbilical
11 arterial blood flow, non-stress test, contraction stress test, and biophysical profile scoring
12 depending on GA, to evaluate possible pregnancy termination. The investigator will evaluate
13 the fetal condition and decide timing of delivery referring to Table 1. For other complications
14 such as preterm labor, rupture of the membranes, and hypertensive disorder of pregnancy, the
15 investigator will follow guidelines for obstetric practice in Japan.¹ The investigator must
16 provide a report that explains the reason for termination of the pregnancy on the website of this
17 clinical trial (the Clinical Trial Data Management System).
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28 **Monitoring Safety during the Fetal Therapy**

29 The investigator must pay close attention to the safety of not only the fetus but also the mother.
30 As shown in the study schedule, the protocol-defined assessments include evaluation of
31 maternal blood pressure and pulse rate, maternal blood and urine tests (blood
32 fibrinogen and anti-thrombin III levels, liver and renal function tests, serum electrolyte levels,
33 and qualitative urine protein excretion), maternal serum placental growth factor (PIGF) and
34 soluble fms-like tyrosine kinase receptor (sFLT-1) levels. Other assessments include adverse
35 events assessed by medical consultation, and antepartum fetal tests consisting of
36 ultrasonography including Doppler imaging of blood flow (umbilical artery, middle cerebral
37 artery, and uterine artery), non-stress test, contraction stress test, and biophysical profile scoring
38 depending on GA. The investigator will enter patients' safety data into the Case Report Form on
39 the website of this clinical trial (the Clinical Trial Data Management System).
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49 **Safety Evaluation Committee**

50 The Safety Evaluation Committee is responsible for the overall safety of this clinical study. To
51 ensure the safety of the protocol-defined treatment, the Safety Evaluation Committee will
52 review the adverse events of tadalafil treatment. If a serious adverse event develops, the
53 investigator will provide the Secretariat with the necessary information within 24 hours of its
54 onset, according to the predetermined procedure. The Secretariat then will forward the obtained
55 information without delay to the Safety Evaluation Committee for review. The Safety
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6 Evaluation Committee will notify the investigator of the review results. If the adverse event is
7 definitely or probably related to tadalafil treatment, the Ethics Committee in Mie University
8 Hospital or each institute will consider possible termination of this clinical study. Special
9 attention must be paid to the reporting requirements stipulated in the Ethical Guidelines for
10 Clinical Studies (Ministry of Health, Labor, and Welfare in Japan, 2017). Infants will be
11 followed up and evaluated for physiological and neurological development until 1.5 years of
12 age.
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19 **Note for New Participating Study Sites**

20 This multicenter study is open to new study sites. It is desirable that study sites cooperate with
21 each other. Agreement on this inter-institutional cooperation is a prerequisite for participation in
22 this clinical study. Case registration requires the approval of the Ethics Committee in each
23 institute.
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5. TREATMENT AND COMPENSATION FOR STUDY-RELATED INJURY

Based on our previous studies, we do not expect that serious adverse events will occur frequently in this study.²² However, the investigator may encounter such adverse events as those mentioned in Section 8: Anticipated Adverse Events. The investigator must report adverse drug reactions to the Minister of Health, Labour and Welfare as provided in the Pharmaceuticals and Medical Devices Act. The investigator must also report any serious adverse events without delay to the head of his or her institution, who will in turn forward the information to the Secretariat. The Secretariat will inform the participating study sites of all reported serious adverse events, irrespective of whether expected or unexpected. The Safety Evaluation Committee will review serious adverse event reports and make recommendations to the Principal Investigator, as appropriate. More specifically, the Safety Evaluation Committee will review the information on a serious adverse event that the investigator forwarded as per the predetermined procedure to the Secretariat within 24 hours of its onset. The Safety Evaluation Committee will notify the review results to the investigator. If the adverse event is definitely or probably related to tadalafil treatment, the Ethics Committee in Mie University Hospital or each institute will consider possible termination of this clinical study. Special attention must be paid to the reporting requirements stipulated in the Ethical Guidelines for Clinical Studies (Ministry of Health, Labor, and Welfare in Japan, 2017).

According to the provisions of the Ethical Guidelines for Clinical Studies (Ministry of Health, Labour and Welfare in Japan, 2017), the study site will inform the Ministry of Health, Labour and Welfare of unexpected adverse events whose study causality cannot be denied. The Ministry of Health, Labour and Welfare will announce reported serious adverse drug reactions to the public at regular intervals. The study site must provide the patient experiencing an adverse event with the most appropriate therapeutic measures available. In this clinical study, maternal complications associated with the protocol-defined treatment have been covered by liability insurance. However, because fetal complications associated with the protocol-defined treatment have not been covered by liability insurance, the investigator must describe this issue in the informed consent document. The corresponding researcher at Mie University is responsible for dealing with inquiries from participating study sites. In case of an accident, the corresponding researcher will consult the Ethics Committee in Mie University for guidance. This study will comply with the reporting requirements provided in the Ethical Guidelines for Clinical Studies (Ministry of Health, Labour and Welfare in Japan, 2017).

6. STUDY PERIOD AND TARGET SAMPLE SIZE

(1) Study Period

The planned study period is from date of ethics approval to February 2021. The Patient Registration Period will last until December 2018. The children's outcome will be followed up for 1.5 years after birth. Data collected by the end of the Neonatal Evaluation Period will be subjected to statistical analysis.

Patient Registration Period: date of ethics approval to December 2018.

Children's Outcome Follow-up Period: 1.5 years after the last birth

(2) Target Sample Size

140 fetuses and their mothers

Rationale for the Target Sample Size

Table 2 shows the summary of the distribution of fetal growth velocity from enrollment to birth in our retrospective study.²¹ We estimate that the distribution of fetal growth velocity of this prospective phase II trial will be similar to that of our retrospective study. When the results of our prospective study are analyzed by Wilcoxon Rank Sum Test and group comparisons, with an α of 0.05, two sided, we will have 90% power to detect a difference if we randomize 62 women per group. Allowing for a 10% drop-out rate, the total sample size required is 140 women.

Table 2. The distribution of fetal growth velocity from enrollment to birth in the retrospective study conducted at Mie University Hospital.

Fetal growth velocity (g/day)	<5	≥5 to <10	≥10 to <15	≥15 to <20	≥20 to <25	≥25
The conventional management group (%)	5.3	10.5	21.1	47.3	15.8	0
The tadalafil group (%)	0	8.3	8.3	50.0	16.7	16.7

7. OUTLINE OF THE STUDY PLAN

1. The investigator will register patients with the Clinical Trial Data Management System according to the procedure defined above.
2. The Clinical Trial Data Management System will register and allocate them to the two arms in an allocation ratio of 1:1, one group receiving the conventional management of FGR according to the guidelines for obstetrical practice in Japan,¹ and the other receiving once-daily treatment with 20 mg tadalafil added to conventional management after adjustment for study sites and GA (<28 or ≥28 weeks of gestation).

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6 3. The investigator will conduct the protocol-defined treatment. The Stopping Criteria and the
7 Criteria for Delivery are explained in detail above.

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9 4. Timing and Methods of Evaluation

10 The investigator will evaluate the variables listed below according to the study schedule. The
11 investigator will use the Case Report Form on the website of this clinical trial (the Clinical Trial
12 Data Management System).

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15 5. Variables

16 The following safety and efficacy variables will be statistically analyzed:

17
18
19 **Variables**

20
21 **(1) Maternal and Fetal**

22 i) Signs and symptoms

23 Headache, vertigo, flushing, epistaxis, palpitations, anorexia, dyspepsia, diarrhea, nausea,
24 myalgia, arthralgia, dyspnea, and fetal movement counting.

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27 ii) Maternal vital signs

28 Blood pressure and pulse rate.

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31 iii) Maternal blood and urine test

32 Complete blood count, blood fibrinogen and anti-thrombin 3 levels, liver and renal function
33 tests, serum electrolyte levels, qualitative urine protein excretion, maternal serum placental
34 growth factor (PIGF), and soluble fms-like tyrosine kinase receptor (sFLT-1) levels.

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37 iv) Fetal ultrasound examination

38 Estimated fetal weight (g), fetal head circumference (cm), deepest amniotic fluid pocket (cm),
39 Doppler imaging of blood flow (umbilical artery, middle cerebral artery, and uterine artery)

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42 v) Obstetrics

43 Onset of obstetrical complications including hypertensive disorder of pregnancy (HDP),
44 treatment for obstetrical complications, indication for delivery, mode of delivery, and placental
45 weight.

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48 vi) Compliance of tadalafil treatment (arm B only).

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51 vi) Adverse events

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53 **(2) Neonatal**

54 i) GA at birth.

55
56 ii) Physical development

57 Body weight, height, head circumference, and percentile of birth weight for GA and sex

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59 iii) Apgar score
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iv) Clinical laboratory testing

Umbilical artery pH and base excess values

v) Admission in the NICU

vi) Neonatal complications

Respiratory distress syndrome (RDS), pulmonary hemorrhage, neonatal pulmonary hypertension, neonatal chronic lung disease, symptomatic patent ductus arteriosus (PDA), late-onset circulatory dysfunction, intraventricular hemorrhage, periventricular leukomalacia, hypoxic-ischemic encephalopathy, sepsis, necrotizing enterocolitis, gastroesophageal reflux, meconium plug syndrome, retinopathy of prematurity (ROP), anemia of prematurity, auditory disorder (abnormal auditory brainstem response results), congenital abnormality, death, and others.

(3) Pediatric

Physiological and neurological developmental assessment until 1.5 years of age, infant complications including cerebral palsy and epilepsy, and death.

Study Endpoints

(1) Primary endpoint

Fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day).

The primary endpoint is fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day), and is calculated using the following formula:

$$\text{Fetal growth velocity (g/day)} = \frac{\text{Birthweight} - \text{EFW at the first day of the treatment [g]}}{\text{Days of the treatment [days]}}$$

Rationale for the primary endpoint

Our primary hypothesis is that tadalafil therapy will increase the likelihood of increased fetal growth velocity in fetuses with FGR. Taking into account the results of our retrospective study demonstrating that tadalafil treatment increased fetal growth velocity (g/day), which was the primary outcome of the retrospective study, and decreased the incidence of RDS, an improvement in fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day) is an important indicator of the therapeutic benefits for fetuses with FGR.²¹

(2) Secondary endpoints

1) Completion rate of the treatment regimen.

Completion rate of the treatment regimen is defined as the percentage of enrolled patients who receive the protocol-defined treatment for more than 7 days.

2) Efficacy endpoints.

i) Estimated fetal weight (g).

Estimated fetal weight (EFW) is calculated using the following formula:²⁷

$$\text{EFW (g)} = 1.07 \times (\text{biparietal diameter: BPD})^3 + 0.3 \times (\text{abdominal circumference: AC})^2 \times (\text{femur length: FL})$$

ii) Fetal growth velocity in the two weeks after the protocol-defined treatment and in the two weeks after one week of the protocol-defined treatment (g/day).

Fetal growth velocity in the two weeks after the protocol-defined treatment (g/day) is calculated using the following formula:

$$\begin{aligned} & \text{Fetal growth velocity in the two weeks after the protocol – defined treatment (g/day)} \\ &= \frac{(\text{EFW two weeks after the treatment} - \text{EFW at the first day of the treatment [g]})}{14 \text{ [days]}} \end{aligned}$$

and fetal growth velocity in the two weeks after one week of the protocol-defined treatment (g/day) is calculated using the following formula:

$$\begin{aligned} & \text{Fetal growth velocity in the two weeks after one week of the treatment (g/day)} \\ &= \frac{(\text{EFW three weeks after the treatment} - \text{EFW one week after the treatment [g]})}{14 \text{ [days]}} \end{aligned}$$

iii) Fetal growth rate in the two weeks after the protocol-defined treatment and from the first day of the protocol-defined treatment to birth (%/day).

Fetal growth rate in the two weeks after the protocol-defined treatment (%/day) is calculated using the following formula:

$$\begin{aligned} & \text{Fetal growth rate in the two weeks after the protocol-defined treatment (%/day)} \\ &= \frac{\frac{\text{EFW two weeks after the treatment} - \text{EFW at the first day of the treatment [g]}}{\text{EFW at the first day of the treatment [g]}} \times 100}{14 \text{ [days]}} \end{aligned}$$

and Fetal growth rate from the first day of the protocol-defined treatment to birth (%/day) is calculated using the following formula:

$$\begin{aligned} & \text{Fetal growth rate from the first day of the protocol-defined treatment to birth (%/day)} \\ &= \frac{\frac{\text{Birthweight} - \text{EFW at the first day of the treatment [g]}}{\text{EFW at the first day of the treatment [g]}} \times 100}{\text{Days of the treatment [days]}} \end{aligned}$$

iv) Fetal head circumference (cm).

The fetal head circumference was measured at the plane of the third ventricle with the thalamus

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6 in the central portion and the cavum septi pellucidi visible in the anterior portion.

7 v) Doppler imaging of umbilical arterial blood flow.

9 Umbilical arterial blood flow was examined by Doppler ultrasound according to the Society for
10 Maternal-Fetal Medicine (SMFM) Clinical Guideline.²⁸

11 vi) Deepest amniotic fluid pocket (cm).

12 The deepest amniotic fluid pocket was measured by transabdominal ultrasonography.

13 vii) Prolongation of gestational age at birth (days).

14 Prolongation of gestational age at birth is defined as days from the first day of the
15 protocol-defined treatment to birth.

16 viii) Birth weight (g).

17 Birth weight is defined as the weight of the infant at birth.

18 ix) GA at birth.

19 GA at birth is defined as the gestational age at birth.

20 x) Apgar score.

21 The Apgar score consists of an evaluation of five factors: heart rate, respiratory effort, muscle
22 tone, responsiveness and color at one minute and five minutes after birth.

23 xi) Umbilical artery pH and base excess values.

24 Umbilical artery pH and base excess is measured at delivery.

25 xii) Incidence rate of pre-eclampsia.

26 Incidence rate of pre-eclampsia is defined as the percentage of enrolled patients who develop
27 pre-eclampsia after the protocol-defined treatment.

28 xiii) Pediatric developmental assessment until 1.5 years of age.

29 Pediatric developmental assessment includes physiological and neurological developmental
30 assessment, and infant complications including cerebral palsy, epilepsy, and death.

31 3) Safety endpoints

32 i) Incidence rate of obstetric complications.

33 Incidence rate of obstetric complications including HDP is defined as the percentage of enrolled
34 patients who develop obstetric complications after the protocol-defined treatment.

35 ii) Perinatal mortality.

36 Perinatal mortality is defined to include stillbirths (occurring after 22 weeks of gestation) and
37 neonatal deaths (occurring up to 7 days after birth).

38 iii) Neonatal mortality.

39 Neonatal mortality is defined as neonatal deaths occurring up to 28 days after birth.

(3) Statistics

Analysis is done on all randomized fetuses who receive the protocol-defined treatment at least once, as the full analysis set. Analysis per protocol set (i.e., removing patients who do not meet the inclusion and exclusion criteria) is done as a secondary analysis population for sensitivity analysis. All outcome measures are presented as summaries of descriptive statistics (mean [SD] or median [minimum and maximum] for continuous measures, and the numbers and proportions for ordinal and dichotomous measures). Descriptive statistics for the primary endpoint, i.e. fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day), are analyzed for each treatment arm by the Wilcoxon Rank Sum Test and group comparisons. All analyses are performed according to a pre-specified statistical analysis plan. The Data Coordinating Center in Mie University Hospital supports the data management, statistical analysis, and reporting of the study.

8. ANTICIPATED ADVERSE EVENTS

Because we have already demonstrated in phase I clinical trial that tadalafil treatment was feasible in pregnant women with FGR,²² tadalafil treatment for FGR can be administered with relative safety and ease. Yet, this therapy may give rise to unexpected adverse events, given the limited clinical experience with this approach and exposure of healthy mothers without pulmonary hypertension to tadalafil. The investigator must fully inform prospective participants of such possibility and administer the fetal therapy with careful attention and monitoring. Adverse reactions to tadalafil divided into the four groups by the frequency (Very common [$\geq 1/10$], common [$\geq 1/100$ to $< 1/10$], uncommon [$\geq 1/1,000$ to $< 1/100$], and not known [cannot be estimated from the available data]) described in the product information of tadalafil (ADCIRCA[®] 20 mg tablets) are shown below:²⁹

- Very common ($\geq 1/10$)
Headache, flushing, nasopharyngitis, nausea, dyspepsia, myalgia, neck pain, and pain in extremity.
- Common ($\geq 1/100$ to $< 1/10$)
Hypersensitivity reactions*, syncope, migraine*, blurred vision, palpitations* ***, hypotension, epistaxis, vomiting, gastroesophageal reflux, rash, increased uterine bleeding**, facial oedema, and chest pain***.
- Uncommon ($\geq 1/1,000$ to $< 1/100$)

Seizures*, transient amnesia*, tinnitus, Sudden cardiac death****, Tachycardia****, hypertension, urticaria*, hyperhidrosis*, haematuria, priapism*, penile haemorrhage, and haemospermia

- Not known (cannot be estimated from the available data)
Angioedema, stroke***, non-arteritic anterior ischemic optic neuropathy, retinal vascular occlusion, visual, field defect, sudden hearing loss, unstable angina pectoris, ventricular arrhythmia, myocardial infarction***, Stevens-Johnson Syndrome, exfoliative dermatitis, and prolonged erections.

* The adverse reactions have been included in the table as a result of postmarketing or clinical study data from the use of tadalafil in the treatment of erectile dysfunction; and in addition, the frequency estimates are based on only 1 or 2 patients experiencing the adverse reaction in the pivotal placebo controlled study of ADCIRCA®.

** Clinical non-Medical Dictionary for Regulatory Activities (MedDRA) term to include reports of abnormal/excessive menstrual bleeding, conditions such as menorrhagia, metrorrhagia, menometrorrhagia, or vaginal hemorrhage.

***Most of the patients in whom these events have been reported had pre-existing cardiovascular risk factors.

9. POTENTIAL BENEFITS AND RISKS

(1) Benefits

Potential benefits of this study include cure or improvement in FGR.

(2) Risks

Maternal exposure to tadalafil is inevitable in patients allocated tadalafil treatment arm. Therefore, precautions must ensure the safety of both the mother and the fetus. Specific descriptions of such risks have been described in Section 8: Anticipated Adverse Events. To control for such risks, this study has stipulated an array of tests, such as hematology, serum chemistry, medical consultation, and antepartum fetal tests consisting of ultrasonography including Doppler imaging of blood flow (umbilical artery, middle cerebral artery, and uterine artery), non-stress test, contraction stress test, and biophysical profile scoring depending on GA. In the event of an adverse drug reaction, the investigator will immediately take appropriate measures, possibly including early withdrawal from the study. The investigator must prioritize maternal safety over fetal therapy. If the mother develops an adverse drug reaction, it will be treated under liability insurance and / or the national health insurance scheme.

10. BURDEN OF COST

This research was supported by by the Japan Agency for Medical Research and Development (AMED). This fund will be paid for items related to research (purchasing cost for tadalafil, data management, storage, analysis, etc.) other than medical examination. Medical examination expenses are covered by the national health insurance scheme.

11. INTELLECTUAL PROPERTY RIGHTS

Any intellectual property rights that may arise from this clinical study shall be exclusively owned by the TADAFER study group. The corresponding researcher and the joint researchers report no conflicts of interest related to this clinical study or to their organizations.

12. ETHICS

This clinical study focuses on prenatal treatment, and its protocol has been developed according to the Ethical Guidelines for Clinical Studies (Ministry of Health, Labour and Welfare in Japan, 2017). Before the start of this clinical study, the corresponding researcher will explain its objectives and outline them fully to the participating site investigators. We believe that application of the guideline requirements to the mother who consents to participate in this study will ensure that her fetus is also protected by the ethical principles of the guidelines. As per the Ethical Guidelines for Clinical Studies, participation in this study will be preceded by the informed consent process. Considering the difficulty in obtaining assent, even implicitly, from the fetus, we believe that the parental permission for the fetus to participate.

13. ADDITIONAL NOTES RELATED TO THE ACT ON THE PROTECTION OF PERSONAL INFORMATION RELATED BY INDEPENDENT ADMINISTRATIVE INSTITUTIONS

1. Data Collection

Study data will be de-identified before being stored in electronic format. De-identified or anonymous data will be analyzed at Mie University. Joint researchers will examine and discuss the analyzed results.

2. Data Management

The results of analyses of the collected test data will be securely stored at the Secretariat located in Mie University.

3. Storage of Electronic Media

The results of analyses will be filed in electronic media, which will be kept securely in a locked room of Mie University. The Secretariat staff member, Dr. Takashi Umekawa, assumes the responsibility for data storage. In addition to the corresponding researcher, appointed members of the Secretariat staff will be granted access to the study data.

4. Method and Timing of Data De-identification

Registration numbers will be used to de-identify the study data at individual study sites. Each study site must ensure that the data they transfer to the Secretariat contains no explicit personal identifiers.

5. Notification of Analytical Results

Parents who participate in this study will not be informed of the results of this study.

14. REFERENCE

- 1 Minakami H, Maeda T, Fujii T, Hamada H, Iitsuka Y, Itakura A *et al.*
2 Guidelines for obstetrical practice in Japan: Japan Society of Obstetrics and Gynecology
3 (JSOG) and Japan Association of Obstetricians and Gynecologists (JAOG) 2014 edition. *J*
4 *Obstet Gynaecol Res* 2014; **40**: 1469–1499.
- 5
6
7
8
9
10
11
12
13
14 2 Kusuda S, Fujimura M, Sakuma I, Aotani H, Kabe K, Itani Y *et al.*
15 Morbidity and mortality of infants with very low birth weight in Japan: center variation.
16 *Pediatrics* 2006; **118**: e1130–e1138.
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4
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6 *Perinatol* 2012; **29**: 429–434.

7 13 Cross JC, Hemberger M, Lu Y, Nozaki T, Whiteley K, Masutani M *et al.*
8 Trophoblast functions, angiogenesis and remodeling of the maternal vasculature in the placenta.
9 *Mol Cell Endocrinol* 2002; **187**: 207–212.

10 14 Watson ED, Cross JC. Development of structures and transport functions in
11 the mouse placenta. *Physiol Bethesda Md* 2005; **20**: 180–193.

12 15 von Dadelszen P, Dwinnell S, Magee LA, Carleton BC, Gruslin A, Lee B *et*
13 *al.* Sildenafil citrate therapy for severe early-onset intrauterine growth restriction. *BJOG Int J*
14 *Obstet Gynaecol* 2011; **118**: 624–628.

15 16 Sharp A, Comforth C, Jackson R, Turner M, Kenny L, Baker P *et al.*
16 OC01.05: STRIDER UK: a randomised controlled trial of sildenafil therapy in dismal prognosis
17 early-onset intrauterine growth restriction. *Ultrasound Obstet Gynecol* 2017; **50**: 3–3.

18 17 Sahni S, Palkar AV, Rochelson BL, Kępa W, Talwar A. Pregnancy and
19 pulmonary arterial hypertension: A clinical conundrum. *Pregnancy Hypertens Int J Womens*
20 *Cardiovasc Health* 2015; **5**: 157–164.

21 18 Wilkins MR, Wharton J, Grimminger F, Ghofrani HA. Phosphodiesterase
22 inhibitors for the treatment of pulmonary hypertension. *Eur Respir J* 2008; **32**: 198–209.

23 19 Fogue ST, Patterson BE, Bedding AW, Payne CD, Phillips DL, Wrishko
24 RE *et al.* Tadalafil pharmacokinetics in healthy subjects. *Br J Clin Pharmacol* 2006; **61**: 280–
25 288.

26 20 Yoshikawa K, Umekawa T, Maki S, Kubo M, Nii M, Tanaka K *et al.*
27 Tadalafil Improves L-NG-Nitroarginine Methyl Ester-Induced Preeclampsia With Fetal Growth
28 Restriction-Like Symptoms in Pregnant Mice. *Am J Hypertens In press.*

29 21 Kubo M, Umekawa T, Maekawa Y, Tanaka H, Nii M, Murabayashi N *et al.*
30 Retrospective study of tadalafil for fetal growth restriction: Impact on maternal and perinatal
31 outcomes. *J Obstet Gynaecol Res* 2017; **43**: 291–297.

32 22 Kubo M, Tanaka H, Maki S, Nii M, Murabayashi N, Osato K *et al.* Safety
33 and dose-finding trial of tadalafil administered for fetal growth restriction: A phase-1 clinical
34 study. *J Obstet Gynaecol Res* 2017; **43**: 1159–1168.

35 23 Cunningham F, Leveno K, Bloom S, Spong CY, Dashe J. *Williams*
36 *Obstetrics, 24e.* McGraw-Hill, 2014.

37 24 Ganzevoort W, Alfirevic Z, von Dadelszen P, Kenny L, Papageorgiou A,
38 van Wassenaer-Leemhuis A *et al.* STRIDER: Sildenafil Therapy In Dismal prognosis
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5
6 Early-onset intrauterine growth Restriction—a protocol for a systematic review with individual
7 participant data and aggregate data meta-analysis and trial sequential analysis. *Syst Rev* 2014; **3**:
8 23.

9
10
11 25 Parer JT, Ikeda T. A framework for standardized management of intrapartum
12 fetal heart rate patterns. *Am J Obstet Gynecol* 2007; **197**: 26–e1.

13
14 26 Shinichi Nishiuma, Kimiko Arakawa, Masanori Taketsuna, Nobuyuki
15 Kobayashi. Safety and effectiveness of tadalafil in patients with erectile dysfunction based on
16 post marketing surveillance study. *Jpn J Impot Res* 2012; **27**: 15–26.

17
18
19 27 Shinozuka N. Fetal biometry and fetal weight estimation: JSUM
20 standardization. *Ultrasound Rev Obstet Gynecol* 2002; **2**: 156–161.

21
22 28 Berkley E, Chauhan SP, Abuhamad A, Committee S for M-FMP. Doppler
23 assessment of the fetus with intrauterine growth restriction. *Am J Obstet Gynecol* 2012; **206**:
24 300–308.

25
26
27 29 ADCIRCA, INN - Tadalafil - WC500032789.pdf.
28 http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/001021/WC500032789.pdf (accessed 19 Nov2017).



Title: TADAFER II: Tadalafil treatment for fetal growth restriction- a study protocol for a multicenter randomized controlled phase II trial.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page No
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Page 2 Line 5-7
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	N/A
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	Page 24 Line 42-46
Funding	4	Sources and types of financial, material, and other support	Page 17 Line 37-41
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Page 17 Line 29-35
	5b	Name and contact information for the trial sponsor	N/A
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			

1	Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	Page 5-7 Line 6-32	
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7	Objectives	6b	Explanation for choice of comparators	N/A	
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9		7	Specific objectives or hypotheses	Page 7 Line 22-26	
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12	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	Page 7 Line 24-26	
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19	Methods: Participants, interventions, and outcomes				
20	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	Page 9 Line 18-44	
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23	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Page 7 Line 50-56 Page 8 Line 21	
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26	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Page 9 Line 31-44	
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31		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	Page 13 Line 19-32	
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34		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	Page 14 Line 5-21	
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39		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Page 9 Line 49-56	
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43	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	Page 10 Line 12-17 Page 13 Line 17	
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1	Participant	13	Time schedule of enrolment, interventions (including any run-ins	Page 12
2	timeline		and washouts), assessments, and visits for participants. A	Line 7-25
3			schematic diagram is highly recommended (see Figure)	
4				
5	Sample size	14	Estimated number of participants needed to achieve study	Page 14
6			objectives and how it was determined, including clinical and	Line 44-
7			statistical assumptions supporting any sample size calculations	Page 15
8				Line 14
9				
10	Recruitment	15	Strategies for achieving adequate participant enrolment to reach	N/A
11			target sample size	
12				
13				

Methods: Assignment of interventions (for controlled trials)

Allocation:

16				
17	Sequence	16a	Method of generating the allocation sequence (eg, computer-	Page 9
18	generation		generated random numbers), and list of any factors for	Line 34-
19			stratification. To reduce predictability of a random sequence,	36
20			details of any planned restriction (eg, blocking) should be	
21			provided in a separate document that is unavailable to those	
22			who enrol participants or assign interventions	
23				
24				
25	Allocation	16b	Mechanism of implementing the allocation sequence (eg,	Page 9
26	concealment		central telephone; sequentially numbered, opaque, sealed	Line 34-
27	mechanism		envelopes), describing any steps to conceal the sequence until	44
28			interventions are assigned	
29				
30				
31	Implementation	16c	Who will generate the allocation sequence, who will enrol	N/A
32			participants, and who will assign participants to interventions	
33				
34	Blinding	17a	Who will be blinded after assignment to interventions (eg, trial	Page 9
35	(masking)		participants, care providers, outcome assessors, data analysts),	Line 21
36			and how	
37				
38		17b	If blinded, circumstances under which unblinding is permissible,	Page 16
39			and procedure for revealing a participant's allocated intervention	Line 43-
40			during the trial	53
41				

Methods: Data collection, management, and analysis

42				
43				
44	Data collection	18a	Plans for assessment and collection of outcome, baseline, and	Page 9
45	methods		other trial data, including any related processes to promote data	line12-24
46			quality (eg, duplicate measurements, training of assessors) and	
47			a description of study instruments (eg, questionnaires,	
48			laboratory tests) along with their reliability and validity, if known.	
49			Reference to where data collection forms can be found, if not in	
50			the protocol	
51				
52				
53		18b	Plans to promote participant retention and complete follow-up,	N/A
54			including list of any outcome data to be collected for participants	
55			who discontinue or deviate from intervention protocols	
56				
57				
58				
59				
60				

1				
2	Data	19	Plans for data entry, coding, security, and storage, including any	Page 50
3	management		related processes to promote data quality (eg, double data	line 49-
4			entry; range checks for data values). Reference to where details	Page 50
5			of data management procedures can be found, if not in the	line 20
6			protocol	
7				
8	Statistical	20a	Statistical methods for analysing primary and secondary	Page 15
9	methods		outcomes. Reference to where other details of the statistical	line 16-35
10			analysis plan can be found, if not in the protocol	
11				
12		20b	Methods for any additional analyses (eg, subgroup and adjusted	N/A
13			analyses)	
14				
15		20c	Definition of analysis population relating to protocol non-	Page 14
16			adherence (eg, as randomised analysis), and any statistical	line 47-
17			methods to handle missing data (eg, multiple imputation)	Page 15
18				line 14
19				
20				
21	Methods: Monitoring			
22				
23	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of	N/A
24			its role and reporting structure; statement of whether it is	
25			independent from the sponsor and competing interests; and	
26			reference to where further details about its charter can be found,	
27			if not in the protocol. Alternatively, an explanation of why a DMC	
28			is not needed	
29				
30		21b	Description of any interim analyses and stopping guidelines,	Page 13
31			including who will have access to these interim results and	line 19-54
32			make the final decision to terminate the trial	
33				
34	Harms	22	Plans for collecting, assessing, reporting, and managing	Page 14
35			solicited and spontaneously reported adverse events and other	line 5-21
36			unintended effects of trial interventions or trial conduct	
37				
38				
39	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and	Page 14
40			whether the process will be independent from investigators and	line 24-42
41			the sponsor	
42				
43	Ethics and dissemination			
44				
45	Research ethics	24	Plans for seeking research ethics committee/institutional review	Page 15
46	approval		board (REC/IRB) approval	Line 37-48
47				
48	Protocol	25	Plans for communicating important protocol modifications (eg,	N/A
49	amendments		changes to eligibility criteria, outcomes, analyses) to relevant	
50			parties (eg, investigators, REC/IRBs, trial participants, trial	
51			registries, journals, regulators)	
52				
53	Consent or assent	26a	Who will obtain informed consent or assent from potential trial	Page 50
54			participants or authorised surrogates, and how (see Item 32)	Line 33-
55				40
56				
57				
58				
59				
60				

1		26b	Additional consent provisions for collection and use of	Page 50
2			participant data and biological specimens in ancillary studies, if	Line 50-
3			applicable	55
4				
5	Confidentiality	27	How personal information about potential and enrolled	Page 51
6			participants will be collected, shared, and maintained in order to	Line 6-13
7			protect confidentiality before, during, and after the trial	
8				
9				
10	Declaration of	28	Financial and other competing interests for principal	Page 17
11	interests		investigators for the overall trial and each study site	Line46-47
12				
13	Access to data	29	Statement of who will have access to the final trial dataset, and	Page 17
14			disclosure of contractual agreements that limit such access for	Line 50-
15			investigators	55
16				
17	Ancillary and	30	Provisions, if any, for ancillary and post-trial care, and for	Page 45
18	post-trial care		compensation to those who suffer harm from trial participation	Line 24-
19				28
20				
21	Dissemination	31a	Plans for investigators and sponsor to communicate trial results	Page 16
22	policy		to participants, healthcare professionals, the public, and other	line25-30
23			relevant groups (eg, via publication, reporting in results	
24			databases, or other data sharing arrangements), including any	
25			publication restrictions	
26				
27				
28		31b	Authorship eligibility guidelines and any intended use of	Page 17
29			professional writers	Line 29-
30				35
31				
32		31c	Plans, if any, for granting public access to the full protocol,	Page 15
33			participant-level dataset, and statistical code	line 44-49
34				
35	Appendices			
36				
37	Informed consent	32	Model consent form and other related documentation given to	Page 37
38	materials		participants and authorised surrogates	Line 37-
39				43
40				
41	Biological	33	Plans for collection, laboratory evaluation, and storage of	N/A
42	specimens		biological specimens for genetic or molecular analysis in the	
43			current trial and for future use in ancillary studies, if applicable	
44				

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