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**Study protocol for a prospective, multicenter, blinded-endpoint phase IV randomized controlled trial (PRIME-V study) regarding the efficacy and safety of ipragliflozin and metformin for visceral fat reduction in patients with type 2 diabetes receiving treatment with dipeptidyl peptidase-4 inhibitors**

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<b>Primary Subject	Diabetes and endocrinology

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Heading</b>:	
Secondary Subject Heading:	Research methods
Keywords:	SGLT-2 inhibitor, metformin, visceral fat reduction, DPP-4 inhibitor, type 2 diabetes, glucose

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5 **Study protocol for a prospective, multicenter, blinded-endpoint phase IV randomized**  
6 **controlled trial (PRIME-V study) regarding the efficacy and safety of ipragliflozin and**  
7 **metformin for visceral fat reduction in patients with type 2 diabetes receiving treatment**  
8 **with dipeptidyl peptidase-4 inhibitors**  
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**ABSTRACT (300 words)**

**Introduction:** In Japan, dipeptidyl peptidase-4 (DPP-4) inhibitors are frequently used as the treatment of choice for patients with type 2 diabetes. In some cases, however, poor glycaemic and body weight control issues persist despite treatment with DPP-4 inhibitors. Previous studies have revealed that sodium-dependent glucose transporter-2 (SGLT-2) inhibitors reduce both plasma glucose levels and body weight in patients with type 2 diabetes. However, the effects of SGLT-2 inhibitors on body composition require further investigation, especially in Asians, who tend to have relatively low-to-moderate body mass indices. Therefore, we aim to determine the effects of treatment with SGLT-2 inhibitors and/or metformin for the reduction of visceral fat in 106 Asian patients with type 2 diabetes undergoing treatment with the DPP-4 inhibitor sitagliptin (50 mg daily) for poor glycaemic control.

**Methods and analysis:** We aim to conduct a prospective, multicenter, blinded-endpoint phase IV randomized controlled trial to evaluate the safety and efficacy of 24 weeks of treatment with either an SGLT-2 inhibitor (ipragliflozin) or metformin for the reduction of visceral fat and plasma glucose levels in patients with type 2 diabetes. Patients who satisfy the eligibility criteria will be randomized (1:1) to receive ipragliflozin (50 mg daily) or metformin (1000 mg daily). The primary outcome is the rate of change in the total area of visceral fat for patients of both groups, as measured using computed tomography, after 24 weeks of therapy. Two radiologists, blinded to the clinical information, will perform centralized analysis of the images in a unified measurement condition.

**Ethics and dissemination:** The protocol was approved by the Institutional Review Board of each hospital. This study is ongoing and due to finish in April 2017. The findings of this study will be disseminated via peer-reviewed publications and conference presentations and will also be disseminated to participants.

**Trial registration:** UMIN000015170

([https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr\\_view.cgi?recptno=R000016861](https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno=R000016861))

**Strengths:**

- The design of this study provides a unique opportunity to examine alternative treatment strategies.
- No studies have compared the effects of SGLT-2 inhibitors and metformin in patients with type 2 diabetes receiving first-line treatment with DPP-4 inhibitors.

**Limitations:**

- This study is not double blind study, however the endpoint evaluation is blinded.

**Keywords:** SGLT-2 inhibitor, metformin, visceral fat reduction, DPP-4 inhibitor, type 2 diabetes, glucose

## INTRODUCTION

Previous studies have estimated that the number of patients with type 2 diabetes mellitus will continue to increase worldwide, especially in Asia.[1, 2] While metformin is regarded as the first-choice treatment for patients with type 2 diabetes in the United States, dipeptidyl peptidase-4 (DPP-4) inhibitors are used by 70% of such patients in Japan for efficacy and safety reasons.[3] Indeed, recent studies indicate that DPP-4 inhibitors are associated with lower risks of hypoglycemia in Asian patients with type 2 diabetes, who tend to have low levels of insulin secretion. Some researchers have also speculated that dietary differences may account for some of the improvements in efficacy.[4-6] In some cases, however, issues with poor glycemic control and body weight control persist despite treatment with DPP-4 inhibitors.[7] In such cases, metformin is recommended as a second-line treatment option. Although numerous studies have supported the efficacy of metformin, which is associated with a low risk of weight gain and reduced cost, the risk of side effects such as gastrointestinal disturbances and severe lactic acidosis often leads to low medication adherence.

Sodium-dependent glucose transporter-2 (SGLT-2) inhibitors have recently been developed, which differ from existing diabetic medications in that they reduce plasma glucose levels by promoting the excretion of glucose through urine.[8] Moreover, research has indicated that SGLT-2 inhibitors also reduce body weight.[9, 10] However, the effects of these medications on body composition remain to be fully elucidated. The reduction of visceral fat is expected to lead to improvements in metabolic syndrome and to prevent the development of atherosclerotic disease. In a previous study, the SGLT-2 inhibitor empagliflozin was observed to exert cardioprotective effects in patients with type 2 diabetes.[11] Adherence to treatment with SGLT-2 inhibitors is expected to be high; however, there is concern that SGLT-2 inhibitors may cause a loss of muscle and bone mass and lead to osteoporosis, as well as decrease in physical function.[10, 12]

Visceral fat obesity has been associated with diabetes, dyslipidemia, and

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5 hypertension.[13] However, reductions in the amount of visceral fat can lead to metabolic  
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7 improvements in patients with diabetes. Previous research indicates that even a 3% reduction in  
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9 body weight has a clinically significant effect on symptoms in obese patients with diabetes.[14]  
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11 However, no studies to date have compared the effects of SGLT-2 inhibitors and metformin on  
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13 visceral fat reduction in patients taking DPP-4 inhibitors.  
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15 A previous study reported that dapagliflozin resulted in body weight reductions in  
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17 patients with type 2 diabetes undergoing treatment with metformin, and that fat accounted for  
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19 two-thirds of this reduction.[10] However, the study investigated a primarily Caucasian  
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21 population. Asian patients with type 2 diabetes have a relatively lower body mass index (BMI)  
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23 relative to Caucasian patients. Therefore, the effects of SGLT-2 inhibitors should be investigated  
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25 in patients with lower BMI.  
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### 30 **Objectives**

31 We aim to conduct a prospective, multicenter, blinded-endpoint phase IV randomized controlled  
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33 trial to evaluate the safety and efficacy of 24 weeks of treatment with either an SGLT-2 inhibitor  
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35 (ipragliflozin) or metformin for the reduction of visceral fat and plasma glucose levels in Asian  
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37 patients with type 2 diabetes (BMI > 22 kg/m<sup>2</sup>) undergoing treatment with the DPP-4 inhibitor  
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39 sitagliptin (50 mg daily) for poor glycemic control. Computed tomography (CT) will be used to  
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41 measure visceral fat at the level of the fourth lumbar vertebra. We will also evaluate the effects  
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43 of each treatment on other metabolic parameters, such as body weight, BMI, blood pressure,  
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45 cholesterol level, waist circumference, bone mineral density, muscle strength, muscle mass, and  
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47 basal metabolism as secondary endpoints.  
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## 52 **METHODS AND ANALYSIS**

### 53 **Study Design**

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55 The PRIME-V study is a randomized, blinded-endpoint study designed and independently  
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5 conducted by Chiba University. The trial organization and a complete list of investigators are  
6 provided in Supplementary Appendix 1. The ethics committee at each participating trial site  
7 approved the protocol and consent form. The study will be conducted in full compliance with  
8 the articles of the Declaration of Helsinki. All analyses will be conducted by Chiba University,  
9 independent of the sponsor and according to the prespecified statistical analysis plan. The first  
10 and second authors wrote the first draft of the manuscript. Executive committee members,  
11 coauthors, and the sponsor will review the data, revise the manuscript, and assume  
12 responsibility for trial adherence to the protocol and the accuracy and completeness of the data  
13 and analyses.  
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### 25 **Sample size calculation**

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27 A total sample size of 106 patients will be required, based on the results of a previous  
28 analysis[15], which reported that SGLT-2 inhibitor treatment produced a 4.0 kg reduction in  
29 body weight and metformin treatment produced a 1.3 kg reduction in body weight. We  
30 calculated that ipragliflozin treatment produces a 20% reduction in visceral fat and metformin  
31 treatment produces a 3% reduction in visceral fat. Assuming a group difference of 17%  
32 (standard deviation (SD)=24.9%), 47 patients per arm will provide >90% power to detect a  
33 difference in rate of visceral fat reduction between ipragliflozin and metformin treatment using  
34 a two-sided, two-sample t-test at a 5% level of significance. Thus, to allow for a 10% dropout  
35 rate, 53 participants are required per group, resulting in a total of 106 participants in the study.  
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### 48 **Recruitment and consent**

49 Recruitment for the present study began in September 2014 and ended in September 2016,  
50 during which time 106 participants were recruited. Participants are currently undergoing  
51 follow-up observation, with the last patient visit due to take place in April 2017. This study is  
52 being conducted at 20 hospitals in Japan. All enrolled patients provided written informed  
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5 consent.  
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### 9 **Eligibility criteria**

#### 10 Inclusion criteria

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12 Eligible patients are those who meet the following inclusion criteria: (a) diagnosis of type 2  
13 diabetes, confirmed in accordance with Japanese guidelines[16] (b) age between 20 and 75  
14 years; (c) inadequate control of plasma glucose levels despite treatment with 50 mg of the  
15 DPP-4 inhibitor sitagliptin for over 12 weeks; (d) HbA1c (glycosylated hemoglobin [Hb], which  
16 provides an indication of the average blood glucose concentration of a patient over the previous  
17 three months) level (according to the National Glycohemoglobin Standardization Program [NGSP])  
18 over 7.0% or under 10.0%; (e) BMI over 22 kg/m<sup>2</sup>; (f) estimated glomerular filtration rate  
19 (eGFR) over 50 mL/min/1.73 m<sup>2</sup>; (g) adequate understanding of the contents of the trial and  
20 provision of written informed consent.  
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#### 33 Exclusion criteria

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35 Patients meeting any of the following exclusion criteria will be excluded from the trial: (a)  
36 diagnosis of type 1 diabetes; (b) history of metabolic acidosis, diabetic coma, and/or pre-coma  
37 up to six months prior to providing consent; (c) history of serious infections requiring insulin  
38 treatment, prior/upcoming surgeries, and/or severe injuries; (d) considerable loss of kidney  
39 function (blood creatinine level over 1.3 mg/dL in men or over 1.2 mg/dL in women) and/or  
40 need for dialysis (including peritoneal dialysis); (e) serious liver damage; (f) history of stroke,  
41 myocardial infarction, heart failure, or other severe cardiovascular complications requiring  
42 hospitalization; (g) use of oral hypoglycemic agents other than DPP-4 inhibitors at the start of  
43 the trial; (h) pregnancy, nursing, or plans to become pregnant; (i) history of chemical sensitivity  
44 to DPP-4 inhibitors, SGLT-2 inhibitors, and/or metformin; (j) current diagnosis of, or at risk for,  
45 urinary tract infection and/or dehydration; (k) positive for ketone bodies; (l) history of lactic  
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5 acidosis; (m) excessive alcohol consumption; (n) history of bone fracture caused by  
6 osteoporosis; (o) need for CT scan within three months prior to providing written consent; (p)  
7 determination of ineligibility by the attending physician for any other reason.  
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### 10 11 12 13 **Random allocation and study medication**

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15 The investigators will send a registration form for an eligible patient to the registration center at  
16 the Chiba University Clinical Trial Data Center (via fax). Registration and allocation will be  
17 implemented at the registration center. Eligible patients who provide written informed consent  
18 will be randomized to treatment with either ipragliflozin (50 mg daily) or metformin  
19 (1000-1500 mg daily) at a ratio of 1:1 by a computer program located at the registration center,  
20 using a minimization method with biased coin assignment balancing of age ( $\leq 65$  or  $> 65$  years  
21 old), HbA1c level ( $\leq 8.0$  or  $> 8.0\%$ ), and waist circumference (men:  $\leq 85$  or  $> 85$  cm; women:  $\leq 80$   
22 or  $> 80$  cm) at the time of screening. (Figure 1.)  
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### 33 **Blinding**

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35 A unified CT imaging condition will be used at all sites and for all participants. Sites will send  
36 electronic imaging data saved using the Digital Imaging and Communication in Medicine  
37 (DICOM) method to the contracted research organization: Micron Inc. (Tokyo, Japan). Micron  
38 will then mask the patients' personal information, such as name, sex, facility, and date of CT  
39 scan, following which the converted data will be sent to two radiologists. The radiologists will  
40 remain blinded to the clinical information and perform centralized analysis of the images in a  
41 unified measurement condition. FatScan® (East Japan Institute of Technology Co., Ltd.,  
42 Ibaraki, Japan) will be used to measure visceral fat area, subcutaneous fat area, total fat area,  
43 waist circumference, and CT level (bone density) of the fourth lumbar vertebra, and  
44 cross-sectional area of abdominal muscle with. The average values will then be calculated.  
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### Interventions

Ipragliflozin or metformin will be administered for 24 weeks. The study medication will be initiated on day 0 after the first CT scan. The metformin dose will be increased to 1000 mg daily at 2 to 4 weeks, provided the patient does not experience adverse gastroenterological effects. The metformin dose will also be increased to 1500 mg daily at 12 weeks, provided HbA1c is  $\geq 7.4\%$  for patients with day 0 HbA1c values  $\geq 8.0\%$  or  $\geq 6.9\%$  for patients with day 0 HbA1c values  $< 8.0\%$ .

### Treatment adherence

To evaluate treatment adherence, the investigators will ask patients how many times each medication was taken during each visit.

### Concomitant medication

Use of additional drugs or therapies such as anti-diabetic agents other than sitagliptin, ipragliflozin, or metformin; anti-obesity medications such as mazindol, cetilistat, or bofu-tsusho-san; and other drugs such as mosapride, ephedrine, or citric acid supplements will not be permitted during the study period. Patients will be instructed not to alter their diet and exercise programs during the study. The use of anti-coagulants, anti-hypertensive agents, anti-dyslipidemia agents, and diuretics will be permitted. However, alterations in medication dose and initiation/termination should be avoided when possible.

### Outcomes

The rate of change in the total area of visceral fat in each group, as measured via CT following the 24-week treatment period, was regarded as the primary outcome. Secondary outcomes including the rates of change in (a) HbA1c (NGSP); (b) body weight and BMI; (c) waist circumference; (d) bone markers (alkaline phosphatase (ALP), bone alkaline phosphatase

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5 (BAP), and tartrate-resistant acid phosphatase-5b (TRACP5b)); (e) muscle strength; (f) fasting  
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7 plasma glucose level, homeostatic model assessment (HOMA)-b, and HOMA-R; (g) cholesterol  
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9 level (total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) as calculated using  
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11 the Friedewald Equation, fasting triglycerides (TG), high-density lipoprotein cholesterol  
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13 (HDL-C)); (h) blood pressure; (i) adipocytokine (adiponectin) and inflammatory markers  
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15 (hs-CRP); (j) subcutaneous fat area and total fat area; (k) respiratory quotient, basal metabolism,  
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17 whole body dual-energy x-ray absorption (DXA), eating behavior questionnaire, calorie and  
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19 glucose intake; (l) area of abdominal muscle as measured via CT; and (m) bone density in the  
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21 fourth lumbar vertebra as measured via CT. Levels of BAP, TRACP5b, insulin, adipocytokine  
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23 (adiponectin), inflammatory markers (hs-CRP), and  $\alpha$ 1-microglobulin will be measured at a  
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25 central laboratory (LSI Medience Corporation, Tokyo, Japan).  
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28        Serious adverse events will be documented and reported according to regulatory  
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30 requirements.  
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### 32 33 **Data collection**

### 34 35 **Study visits and examinations**

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37 The schedule for the study visits and data collection is summarized in Table 1.  
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**Table 1.** Schedule of data collection

	Before observation period	Administration start	Dosing period				
			2 weeks	4 weeks	8 weeks	12 weeks	24 weeks
	Within 4 weeks	Day 0					
Allowance			Within $\pm$ 1 week	Within $\pm$ 1 week	Within $\pm$ 2 weeks	Within $\pm$ 2 weeks	Within $\pm$ 4 weeks
Visit	1	2	3	4	5	6	7
Informed consent	X						
Patient characteristics	X						
Study drug administration		←————— X —————→					
Symptom check		←————— X —————→					
Adverse events check		←————— X —————→					
Visceral fat area measured via CT		X					X
Body weight	X	X	X	X	X	X	X
Heart rate, blood pressure		X	X	X	X	X	X
Blood tests		X	X	X	X	X	X
Blood chemistry		X	X	X	X	X	X
Urine tests		X	X	X	X	X	X
Insulin, bone marker, inflammation marker		X				X	X
$\alpha$ 1-microglobulin		X					X
Adipocytokine		X					X
Waist circumference	X	X	X	X	X	X	X
Hand griping test		X				X	X
Medication adherence check			X	X	X	X	X
Special examination		X					X
Screening blood examination	X						

### **Data management, monitoring, and auditing**

The investigators (or their delegates) will maintain individual records for each patient as source data, which will include a log of informed consent, medical records, laboratory data, and other records or notes, as appropriate. All entries in the case report form (CRF) must be backed up by the relevant source data. CRFs must be completed in a timely manner.

All data will be collected by the independent data management center. There will be no direct communication between investigators and the coordinating data center. The clinical data entry (double data entry), coding, data management, and reporting will be performed using the data management system ACRess (Fujitsu, Tokyo, Japan).

A monitor will confirm that the investigational team is adhering to the protocol, that data are being accurately recorded in CRFs, that adverse events (AEs) have been properly documented on CRFs, that severe AEs (SAEs) have been forwarded to the coordinating investigator and the provider of the investigational product, and that the SAEs that met criteria for reporting have been forwarded to the Institutional Review Board (IRB). An interim analysis will not be performed.

The study may be audited or inspected by a third party: Increase Co., Ltd. (Tokyo, Japan). In case of an audit, the investigators must make all study documentation available to the auditor. If an audit or inspection occurs, the investigators at the study site must discuss the findings and any relevant issues.

### **Adverse events**

Investigators must record all AEs in the patients' CRFs. All AEs are to be followed up continually during their course until the end of the trial. All SAEs must be reported to all investigators and discussed.

### Statistical analysis

The analyses of the primary and secondary efficacy endpoints will be performed using the full analysis set. Safety analysis will be conducted in the safety analysis population. For the baseline variables, summary statistics will be constructed using frequencies and proportions for categorical data, and means and SDs for continuous variables. Patient characteristics will be compared using Pearson's chi-square test or Fisher's exact test for categorical outcomes, and Student's t-test for continuous variables, as appropriate.

For the primary analysis, in which we aim to evaluate treatment efficacy, the least square mean difference in the rate of visceral fat reduction between ipragliflozin and metformin treatment at week 24 and its 95% confidential interval (CI) will be estimated using an analysis of covariance (ANCOVA) model adjusted for allocation factors (age, HbA1c, abdominal circumference). As a sensitivity analysis, a mixed-effects model for repeated measures (MMRM), the last observational carried forward (LOCF) method, and the multiple imputation method will be applied to examine the effect of missing data. The secondary analysis will be performed in the same manner as the primary analysis. Data regarding hypoglycemia, dehydration, urinary tract infection, drug eruption, and other adverse events (AEs) will be evaluated during the safety analysis. The frequencies of AEs will be compared using Fisher's exact test. A sub-group analysis based on patient characteristics such as diabetes duration, drug combinations, age, BMI, etc. will be performed to investigate mechanisms underlying patient responses to ipragliflozin.

All comparisons have been planned, and all p values will be two-sided. P values of <0.05 will be considered statistically significant. All statistical analyses will be performed using SAS Version 9.4 (SAS Institute, Cary, North Carolina, USA). This plan for statistical analysis was developed by the chief investigator and statisticians at Chiba University, and will be finalized prior to database lock.



## ETHICS AND DISSEMINATION

### Research ethics approval and protocol amendments

Substantial amendments of the study protocol must be approved by the IRB. The study was registered in the UMIN Clinical Trials Registry (UMIN000015170).

### Informed consent

All participants will receive adequate information about the nature, purpose, possible risks and benefits of the trial, and alternative therapeutic choices using an informed consent protocol approved by the IRB. All participants must be given ample time and opportunity to ask questions and to consider participation in the trial. A completed informed consent form is required for enrolment in the trial. The investigators must maintain the original signed consent form, as well as an additional copy of this form.

### Confidentiality

To ensure confidentiality, trial participants will be allocated a unique trial identification number for use throughout the trial.

### Dissemination

The findings of this trial will be disseminated via peer-reviewed publications and conference presentations and will also be disseminated to participants.

## DISCUSSION

In this study, we evaluated the safety and efficacy of 24 weeks of treatment with either an SGLT-2 inhibitor (ipragliflozin) or metformin for the reduction of visceral fat and plasma glucose levels in Asian patients with type 2 diabetes (BMI > 22 kg/m<sup>2</sup>) undergoing treatment with the DPP-4 inhibitor sitagliptin (50 mg daily) for poor glycemic control. We also evaluated

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5 the effects of each treatment on other metabolic parameters. As no studies have compared the  
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7 effects of SGLT-2 inhibitors and metformin on visceral fat reduction in patients with type 2  
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9 diabetes receiving first-line treatment with DPP-4 inhibitors, the design of the present study  
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11 provides a unique opportunity to examine alternative treatment strategies in an Asian  
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13 population.

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15 Clinicians must remain conscious of weight gain following increases in insulin secretion  
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17 when treating patients with type 2 diabetes. Metformin allows for reductions in plasma glucose  
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19 levels without affecting insulin secretion by pancreatic beta cells. In addition to promoting  
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21 uptake in peripheral tissues (mainly muscle) and improving insulin sensitivity, metformin is  
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23 associated with a low risk of body weight gain. Previous studies have further revealed that  
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25 combined treatment with metformin and a DPP-4 inhibitor leads to significant reductions in  
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27 body weight.[17] Therefore, metformin may be an effective second-line treatment option in  
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29 patients with symptoms refractory to treatment with DPP-4 inhibitors. However, metformin has  
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31 been known to induce gastrointestinal disturbances and severe lactic acidosis in some patients.  
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33 Furthermore, the need to take medication two to three times per day often results in poor  
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35 medication adherence.  
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38 Similarly, SGLT-2 inhibitors do not affect insulin secretion. SGLT-2 inhibitors act to  
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40 reduce glucose reabsorption in the kidneys, thereby preventing increases in blood glucose  
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42 levels, reducing the burden of pancreatic beta cells, restoring insulin secretion, and improving  
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44 glucose toxicity and insulin resistance.[8] Clinical studies have reported that treatment with  
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46 SGLT-2 is associated with improvements in insulin sensitivity [18] as well as reductions in body  
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48 weight.[9, 20] In one clinical study, combined treatment with dapagliflozin and metformin  
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50 produced significant reductions in visceral fat.[10] These findings indicate that such treatment  
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52 may aid in lowering the risk of several conditions associated with high levels of visceral fat,  
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54 such as arteriosclerosis. Furthermore, once-daily administration is sufficient, which may  
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56 increase medication adherence.  
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5 The EMPA-REG OUTCOME study also reported that empagliflozin exerts  
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7 cardioprotective effects.[11] Thus, our findings may provide further evidence regarding the  
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9 cardioprotective effects of SGLT-2 inhibitors.  
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11 Further studies have revealed that adjunct treatment with dapagliflozin in patients with  
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13 symptoms refractory to DPP-4 inhibitor treatment resulted in HbA1c reductions of 0.5% and  
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15 body weight reductions of 2.1 kg.[21] In a comparative study of a single treatment with either  
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17 empagliflozin or metformin, metformin treatment resulted in HbA1c reductions of 0.56% and  
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19 body weight reductions of 1.3 kg at 90 weeks after administration, while empagliflozin  
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21 treatment resulted in HbA1c reductions of 0.63% and body weight reductions of 4.0 kg.[15] In  
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23 comparative study of single treatment with either ipragliflozin (50 mg) or metformin (up to  
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25 1500 mg), no significant differences in HbA1c were observed at 12 weeks, although  
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27 ipragliflozin treatment resulted in body weight reductions of 0.78 kg.[22] Based on these  
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29 previous findings, we speculate that combined treatment with an SGLT-2 inhibitor and a DPP-4  
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31 inhibitor results in comparable reductions in blood glucose level and greater visceral fat  
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33 reduction than combined treatment with metformin and a DPP-4 inhibitor. Previous studies have  
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35 provided a strong rationale for dual therapy with a DPP-4 inhibitor and an SGLT-2 inhibitor.[23]  
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37 This study aims to provide new insight on the most appropriate combination of DPP-4 and  
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39 SGLT-2 inhibitors, which may lead to the development of new treatment options for patients  
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41 with type 2 diabetes.  
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44 Although reductions in visceral fat are important for reducing the impact of metabolic  
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46 disorders and preventing complications such as atherosclerosis, no studies have compared the  
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48 effects of SGLT-2 inhibitors and metformin on visceral fat reduction in patients taking DPP-4  
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50 inhibitors. Administration of SGLT-2 inhibitors, particularly in patients with poor glycemic  
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52 control despite treatment with DPP-4 inhibitors, may exert such beneficial effects. However, the  
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54 risk of sarcopenia and osteopenia remains a concern. Therefore, it is necessary to clarify specific  
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56 changes in body composition, rather than reductions in body weight alone, in order to evaluate  
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5 such risks. Our study will provide evidence regarding the safety and efficacy of the SGLT-2  
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7 inhibitor ipragliflozin as a second-line treatment for the reduction of visceral fat and blood  
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9 glucose levels in patients with type 2 diabetes.  
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14  
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16  
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18  
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22  
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26  
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28  
29 English language editing.  
30  
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36  
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38  
39

### 40 41 **Competing interests**

42  
43 KY received research grants from Astellas Pharma Inc. and MSD K.K. received a lecture fee  
44  
45 from Astellas Pharma Inc. and Sumitomo Dainippon Pharma. No conflicts of interest are  
46  
47 declared for other authors.  
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### 50 51 **Ethics approval**

52  
53 The protocol was approved by the Institutional Review Board of each participating hospital.  
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**Author Contributions**

All authors made a significant contribution to the conception and design of the study protocol. YK designed the original concept. The protocol was written by MK, KI, TI, KK and MT, and it was critically reviewed by TH, RS, ST, KN, YS, IT, TT, NH, NK, DU and KY. All authors gave approval for the publication of the manuscript.

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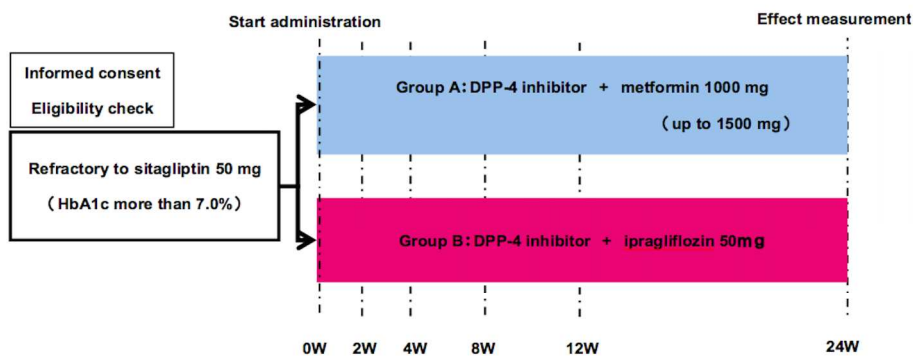


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5 **Figure Legends**  
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7 **Figure 1.** Schematic depiction of the trial design. Eligible participants are randomly assigned to  
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9 a 24-week treatment regimen with either ipragliflozin (50 mg daily) or metformin (1000 mg  
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11 daily, up to 1500 mg).  
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**SUPPLEMENTARY APPENDIX 1****Collaborators**

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Sannou Hospital: Ryouta Shimousa.

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Chihiro Yoneda, Kana Tajima.

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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

Section/item	Item No	Description	Addressed on page number
<b>Administrative information</b>			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	<u>1</u>
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	<u>1</u>
	2b	All items from the World Health Organization Trial Registration Data Set	<u>4-7</u>
Protocol version	3	Date and version identifier	<u>1</u>
Funding	4	Sources and types of financial, material, and other support	<u>4, 57, 58</u>
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	<u>Attachment 3-7</u>
	5b	Name and contact information for the trial sponsor	<u>4, 57, Attachment 5</u>
	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	<u>4, 57, Attachment 5</u>
	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)	<u>Attachment 3-7</u>

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49**Introduction**

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	<u>18-23</u>
	6b	Explanation for choice of comparators	<u>18-23</u>
Objectives	7	Specific objectives or hypotheses	<u>23</u>
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)	<u>29</u>

**Methods: Participants, interventions, and outcomes**

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	<u>Attachment 5.6</u>
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)	<u>28, 29</u>
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	<u>29-34</u>
	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)	<u>32</u>
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	<u>34, 40</u>
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	<u>33, 34</u>
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	<u>35-38</u>
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	<u>31</u>

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3	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	<u>53</u>
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6	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	<u>34, 35,</u> <u>Attachment 5</u>
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### Methods: Assignment of interventions (for controlled trials)

#### Allocation:

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13	Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	<u>6, 29, 30,</u> <u>Attachment 4</u>
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19	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	<u>29, 30, Attachment</u> <u>4</u>
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23	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	<u>Attachment 4</u>
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26	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	<u>6, 39, 43</u>
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29		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	<u>6, 39, 43</u>
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### Methods: Data collection, management, and analysis

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35	Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	<u>35-38, 43, 44</u>
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40		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	<u>32, 45</u>
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Data management 19 Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol 50, 51

Statistical methods 20a Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol 52-55

20b Methods for any additional analyses (eg, subgroup and adjusted analyses) 55

20c Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) 52, 55

**Methods: Monitoring**

Data monitoring 21a Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed 47

21b Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial 49, 50

Harms 22 Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct 45-47

Auditing 23 Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor 51, 52

**Ethics and dissemination**

Research ethics approval 24 Plans for seeking research ethics committee/institutional review board (REC/IRB) approval 58, 59

Protocol amendments 25 Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) 58, 59





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3	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	<u>29</u>
4				
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6		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	<u>56</u>
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9	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	<u>55, 56</u>
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12	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	<u>57, 58</u>
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15	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	<u>55</u>
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18	Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	<u>56, 57</u>
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21	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	<u>57</u>
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26		31b	Authorship eligibility guidelines and any intended use of professional writers	<u>57</u>
27				
28		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	<u>57</u>
29				
30	<b>Appendices</b>			
31				
32	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	<u>Consent form</u>
33				
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35	Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	<u>56</u>
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\*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.

# BMJ Open

## Efficacy and safety of ipragliflozin and metformin for visceral fat reduction in type 2 diabetes patients receiving treatment with dipeptidyl peptidase-4 inhibitors in Japan: A study protocol for a prospective, multicenter, blinded-endpoint phase IV randomized controlled trial (PRIME-V study)

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<b>Primary Subject	Diabetes and endocrinology

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Heading	
Secondary Subject Heading:	Research methods, Nutrition and metabolism
Keywords:	metformin, visceral fat reduction, type 2 diabetes, glucose, sodium-dependent glucose transporter-2 inhibitor, dipeptidyl peptidase-4 inhibitor

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5 **Efficacy and safety of ipragliflozin and metformin for visceral fat reduction in type 2**  
6 **diabetes patients receiving treatment with dipeptidyl peptidase-4 inhibitors in Japan: A**  
7 **study protocol for a prospective, multicenter, blinded-endpoint phase IV randomized**  
8 **controlled trial (PRIME-V study)**  
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5 **1 ABSTRACT (296 words)**

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7 **2 Introduction:** In Japan, dipeptidyl peptidase-4 (DPP-4) inhibitors are frequently used as the  
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treatment of choice for patients with type 2 diabetes. In some cases, however, poor glycaemic  
and body weight control issues persist despite treatment with DPP-4 inhibitors. Previous  
researchers have revealed that sodium-dependent glucose transporter-2 (SGLT-2) inhibitors  
reduce both plasma glucose levels and body weight in patients with type 2 diabetes. However,  
further investigation regarding the effects of SGLT-2 inhibitors on body composition, especially  
in the Asian population that tends to have relatively low-to-moderate body mass indices, is  
required. Therefore, we aim to determine the effects of treatment with SGLT-2 inhibitors or  
metformin for reducing visceral fat in 106 Asian patients with type 2 diabetes who were  
undergoing treatment with the DPP-4 inhibitor sitagliptin (50 mg daily) for poor glycaemic  
control.

13 **13 Methods and analysis:** A prospective, multicenter, blinded-endpoint phase IV randomized  
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controlled trial will be conducted to evaluate the safety and efficacy of a 24-week treatment  
with either an SGLT-2 inhibitor (ipragliflozin) or metformin for reducing visceral fat and plasma  
glucose levels in patients with type 2 diabetes. Patients who satisfy the eligibility criteria will be  
randomized (1:1) to receive ipragliflozin (50 mg daily) or metformin (1000 mg daily). The  
primary outcome is the rate of change in the total area of visceral fat for patients in both  
treatment groups, measured using computed tomography, after 24 weeks of therapy. Two  
radiologists, blinded to the clinical information, will perform centralized analysis of the images  
in a unified measurement condition.

22 **22 Ethics and dissemination:** The protocol was approved by the Institutional Review Board of  
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each hospital. This study is ongoing and due to finish in April 2017. The findings of this study  
will be disseminated via peer-reviewed publications and conference presentations, and will also  
be disseminated to participants.

26 **26 Trial registration:** UMIN000015170

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5 27 ([https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr\\_view.cgi?recptno=R000016861](https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno=R000016861))  
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10 **Strengths:**

- 11 ● The design of this study provides a unique opportunity to examine alternative treatment  
12 strategies.  
13  
14 ● No studies have been conducted to compare the effects of SGLT-2 inhibitors and  
15 metformin in patients with type 2 diabetes receiving first-line treatment with DPP-4  
16 inhibitors.  
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18 ● Computed tomography will be used to measure visceral fat.  
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23 **Limitations:**

- 24 ● This study is not a double-blind study; however, the endpoint evaluation is blinded.  
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29 **Keywords:** sodium-dependent glucose transporter-2 inhibitor, metformin, visceral fat reduction,  
30 dipeptidyl peptidase-4 inhibitor, type 2 diabetes, glucose  
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56 **INTRODUCTION**  
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5 53 Previous researchers have estimated that the number of patients with type 2 diabetes mellitus will  
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7 54 continue to increase worldwide, especially in Asia.[1, 2] While metformin is regarded as the  
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9 55 first-choice treatment for patients with type 2 diabetes in the United States, dipeptidyl  
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11 56 peptidase-4 (DPP-4) inhibitors are used by 70% of such patients in Japan for efficacy and safety  
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13 57 reasons.[3] Indeed, it has been indicated that DPP-4 inhibitors are associated with lower risks of  
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15 58 hypoglycemia in Asian patients with type 2 diabetes who tend to have low insulin secretion  
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17 59 levels. Some researchers have also speculated that dietary differences may account for some of  
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19 60 the efficacy of DPP-4 inhibitors in Asian patients.[4-6] In some cases, however, issues with poor  
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21 61 glycemic control and body weight control persist despite treatment with DPP-4 inhibitors.[7] In  
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23 62 such cases, metformin is recommended as a second-line treatment option. Although the efficacy  
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25 63 of metformin, which is associated with a low risk of weight gain and reduced cost, has been  
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27 64 supported in numerous studies, the risk of side effects (i.e., gastrointestinal disturbances and  
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29 65 severe lactic acidosis) often leads to low medication adherence.

30  
31 66 Sodium-dependent glucose transporter-2 (SGLT-2) inhibitors have recently been  
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33 67 developed, which differ from existing diabetic medications in that they reduce plasma glucose  
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35 68 levels by promoting glucose excretion in urine.[8] Moreover, researchers have indicated that  
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37 69 SGLT-2 inhibitors also reduce body weight.[9, 10] However, the effects of these medications on  
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39 70 body composition need to be fully elucidated. The reduction of visceral fat is expected to lead to  
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41 71 improvements in metabolic syndrome and prevention of atherosclerotic disease. In a previous  
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43 72 study, the SGLT-2 inhibitor, empagliflozin, was observed to exert cardioprotective effects in  
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45 73 patients with type 2 diabetes.[11] Adherence to treatment with SGLT-2 inhibitors is expected to  
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47 74 be high; however, there is concern that SGLT-2 inhibitors may cause a loss of muscle and bone  
48  
49 75 mass and lead to osteoporosis and decreased physical function.[10, 12]

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51 76 Visceral fat obesity has been associated with diabetes, dyslipidemia, and  
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53 77 hypertension.[13] However, reductions in the amount of visceral fat can lead to metabolic  
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55 78 improvements in patients with diabetes. It has been previously indicated that even a 3%  
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5 79 reduction in body weight has a clinically significant effect on the symptoms of obese patients  
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7 80 with diabetes.[14] However, no studies have been conducted to date to compare the effects of  
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9 81 SGLT-2 inhibitors and metformin on visceral fat reduction in patients taking DPP-4 inhibitors.

10  
11 82 In a previous study, treatment with dapagliflozin and metformin resulted in body weight  
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13 83 reductions, which accounted for a 2/3 reduction in fat, in patients with type 2 diabetes.[10]  
14  
15 84 However, this study was conducted primarily in a Caucasian population. Asian patients with  
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17 85 type 2 diabetes have a relatively lower body mass index (BMI) relative to Caucasian patients.  
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19 86 Therefore, the effects of SGLT-2 inhibitors should be investigated in patients with lower BMI.  
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## 22 88 **Objectives**

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25 89 We aim to conduct a prospective, multicenter, blinded-endpoint phase IV randomized controlled  
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27 90 trial (PRIME-V study) to evaluate the safety and efficacy of a 24-week treatment with either an  
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29 91 SGLT-2 inhibitor (ipragliflozin) or metformin for reducing visceral fat and plasma glucose  
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31 92 levels in Asian patients with type 2 diabetes (BMI >22 kg/m<sup>2</sup>) undergoing treatment with the  
32  
33 93 DPP-4 inhibitor, sitagliptin, (50 mg daily) for poor glycemic control. Computed tomography  
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35 94 (CT) will be used to measure visceral fat at the level of the fourth lumbar vertebra. We will also  
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37 95 evaluate the effects of each treatment on other metabolic parameters, such as body weight, BMI,  
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39 96 blood pressure, cholesterol level, waist circumference, bone mineral density, muscle strength,  
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41 97 muscle mass, and basal metabolism as secondary endpoints.  
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## 45 99 **METHODS AND ANALYSIS**

### 46 100 **Study design**

47  
48 101 The PRIME-V study is designed and independently conducted by Chiba University. The trial  
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50 102 organization and a complete list of investigators are provided in Supplementary Appendix 1.  
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52 103 The ethics committee at each participating trial site approved the protocol and consent form.  
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54 104 The study will be conducted in full compliance with the articles of the Declaration of Helsinki.  
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5 105 All analyses will be conducted by Chiba University, independent of the sponsor, according to  
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7 106 the prespecified statistical analysis plan. The first and second authors wrote the first draft of the  
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9 107 manuscript. Executive committee members, coauthors, and the sponsor will review the data,  
10  
11 108 revise the manuscript, and assume responsibility for trial adherence to the protocol and the  
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13 109 accuracy and completeness of the data and analyses.  
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### 111 **Sample size calculation**

112 A total sample size of 106 patients will be required, based on the results of a previous  
113 analysis[15], which reported that SGLT-2 inhibitor and metformin treatments resulted in a  
114 4.0-kg and a 1.3-kg reduction in body weight, respectively. We calculated that ipragliflozin and  
115 metformin treatments produce a 20% and 3% reduction in visceral fat, respectively. Assuming a  
116 group difference of 17% (standard deviation (SD)=24.9%), allocating 47 patients per group will  
117 provide >90% power to detect a difference in the rate of visceral fat reduction between  
118 ipragliflozin and metformin treatment using a two-sided, two-sample, t-test at a 5% level of  
119 significance. To allow for a 10% dropout rate, 53 participants are required per group, resulting  
120 in a total of 106 participants in the study.  
121

### 122 **Recruitment and consent**

123 From September 2014 to September 2016, 106 participants were recruited. Participants are  
124 currently undergoing follow-up observation; the last patient visit is scheduled in April 2017.  
125 This study is being conducted at 20 hospitals in Japan. All enrolled patients provided written  
126 informed consent.  
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### 128 **Eligibility criteria**

#### 129 Inclusion criteria

130 Eligible patients are those who meet the following inclusion criteria: (a) diagnosis of type 2

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5 131 diabetes, confirmed in accordance with Japanese guidelines[16]; (b) age between 20 and 75  
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7 132 years; (c) inadequate control of plasma glucose levels despite treatment with 50 mg of the  
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9 133 DPP-4 inhibitor sitagliptin for >12 weeks; (d) glycosylated hemoglobin (HbA1c, which  
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11 134 provides an indication of the average blood glucose concentration of a patient over the previous  
12  
13 135 3 months) level >7.0% or <10.0% (according to the National Glycohemoglobin Standardization  
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15 136 Program [NGSP]); (e) BMI >22 kg/m<sup>2</sup>; (f) estimated glomerular filtration rate >50 mL/min/1.73  
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17 137 m<sup>2</sup>; and (g) an adequate understanding of the contents of the trial and provision of written  
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19 138 informed consent.  
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23 140 Exclusion criteria

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25 141 Patients meeting any of the following criteria will be excluded from the trial: (a) diagnosis of  
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27 142 type 1 diabetes; (b) history of metabolic acidosis, diabetic coma, and/or pre-coma up to 6  
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29 143 months prior to providing consent; (c) history of serious infections requiring insulin treatment,  
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31 144 prior/upcoming surgeries, and/or severe injuries; (d) considerable loss of kidney function (blood  
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33 145 creatinine level >1.3 mg/dL in men or >1.2 mg/dL in women) and/or need for dialysis  
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35 146 (including peritoneal dialysis); (e) serious liver damage; (f) history of stroke, myocardial  
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37 147 infarction, heart failure, or other severe cardiovascular complications requiring hospitalization;  
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39 148 (g) use of oral hypoglycemic agents other than DPP-4 inhibitors at the start of the trial; (h)  
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41 149 pregnancy, nursing, or plans to become pregnant; (i) history of chemical sensitivity to DPP-4  
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43 150 inhibitors, SGLT-2 inhibitors, and/or metformin; (j) current diagnosis of, or at risk for, urinary  
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45 151 tract infection and/or dehydration; (k) positive for ketone bodies; (l) history of lactic acidosis;  
46  
47 152 (m) excessive alcohol consumption; (n) history of bone fracture caused by osteoporosis; (o)  
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49 153 need for CT scan within 3 months prior to providing written consent; and/or (p) determination  
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51 154 of ineligibility by the attending physician for any other reason.  
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56 156 **Study setting**  
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5 157 The community clinics and academic hospitals in Japan that were involved with this study are  
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7 158 mentioned in Supplementary Appendix 1. Each clinical center involved in this study was chosen  
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9 159 based on patient availability.  
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### 13 161 **Random allocation and study medication**

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15 162 The investigators will send a registration form for an eligible patient to the registration center at  
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17 163 the Chiba University Clinical Trial Data Center (via fax). Registration and allocation will be  
18  
19 164 implemented at the registration center. Eligible patients who provide written informed consent  
20  
21 165 will be randomized to treatment with either ipragliflozin (50 mg daily) or metformin (1000–  
22  
23 166 1500 mg daily) at a ratio of 1:1 by a computer program located at the registration center using a  
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25 167 minimization method with biased coin assignment balancing age ( $\leq 65$  or  $> 65$  years old), HbA1c  
26  
27 168 level ( $\leq 8.0$  or  $> 8.0\%$ ), and waist circumference (men:  $\leq 85$  or  $> 85$  cm; women:  $\leq 80$  or  $> 80$  cm)  
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29 169 at the time of screening (Figure 1).  
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### 33 171 **Visceral fat CT measurement**

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35 172 CT was used measure the visceral, subcutaneous, and total fat areas. The CT images are  
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37 173 measured as the central measurement by 2 blind radiologists and the average value is calculated.  
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39 174 The following imaging conditions will be used at all sites and for all participants: unified CT  
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41 175 imaging; conventional method; voltage 120 kVp; dose 200 mAs; abdominal simple image  
42  
43 176 reconstruction condition; field of view 500 mm; expiratory phase end position for respiratory  
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45 177 phase; and at the fourth lumbar spine center level. The imaging position is the same in all the  
46  
47 178 periods. To minimize exposure to radiation by positioning with scouts, the number of images  
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49 179 obtained should be as minimal as possible. Slice width was preferably 10 mm, or 8 mm if  
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51 180 there are equipment restrictions. For facilities with multiple CT devices, one particular CT  
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53 181 device was used for this study.  
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5 183 **Blinding**  
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7 184 Participating sites will send electronic imaging data saved using the Digital Imaging and  
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9 185 Communication in Medicine method to the contracted research organization (Micron  
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11 186 Technology, Inc.; Tokyo, Japan). Micron Technology, Inc. will then mask the patients' personal  
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13 187 information (i.e., age, sex, facility, and date of CT scan) and send the converted data to 2  
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15 188 radiologists. The radiologists will remain blinded to the clinical information and perform  
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17 189 centralized analysis of the images in a unified measurement condition. FatScan® (East Japan  
18  
19 190 Institute of Technology Co., Ltd., Ibaraki, Japan) will be used to measure the visceral,  
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21 191 subcutaneous, and total fat areas, waist circumference, CT level (bone density) of the fourth  
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23 192 lumbar vertebra, and cross-sectional area of abdominal muscle. The average values for the  
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25 193 above measurements will then be calculated.  
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30 195 **Interventions**

31 196 Ipragliflozin or metformin will be administered for 24 weeks. The study medication will be  
32  
33 197 initiated on day 0 after the first CT scan. The metformin dose will be increased to 1000 mg daily  
34  
35 198 at 2 to 4 weeks, if the patient does not experience adverse gastroenterological effects. The  
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37 199 metformin dose will also be increased to 1500 mg daily at 12 weeks if the HbA1c value is  
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39 200  $\geq 7.4\%$  or  $\geq 6.9\%$  for patients with day 0 HbA1c values  $\geq 8.0\%$  or  $< 8.0\%$ , respectively.  
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44 202 **Treatment adherence**

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46 203 To evaluate treatment adherence, the investigators will ask patients regarding the frequency of  
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48 204 medication use during each visit.  
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52 206 **Concomitant medication**

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54 207 Use of additional drugs or therapies (i.e., anti-diabetic agents other than sitagliptin, ipragliflozin,  
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56 208 or metformin; anti-obesity medications, such as mazindol, cetilistat, or bofu-tsusho-san; and  
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5 209 other drugs, such as mosapride, ephedrine, or citric acid supplements) will not be permitted  
6  
7 210 during the study period. Patients will be instructed not to alter their diet and exercise programs  
8  
9 211 during the study. The use of anti-coagulants, anti-hypertensive agents, anti-dyslipidemia agents,  
10  
11 212 and diuretics will be permitted. However, alterations in medication dose and  
12  
13 213 initiation/termination should be avoided when possible.  
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## 18 215 **Outcomes**

19  
20 216 The rate of change in the total area of visceral fat in each group, as measured via CT  
21  
22 217 following the 24-week treatment period, was regarded as the primary outcome. Secondary  
23  
24 218 outcomes including the rates of change in (a) HbA1c (NGSP); (b) body weight and BMI; (c)  
25  
26 219 waist circumference; (d) bone markers (alkaline phosphatase, bone alkaline phosphatase (BAP),  
27  
28 220 and tartrate-resistant acid phosphatase-5b (TRACP5b); (e) muscle strength; (f) fasting plasma  
29  
30 221 glucose level, homeostatic model assessment (HOMA)-b, and HOMA-R; (g) cholesterol level  
31  
32 222 (total cholesterol, low-density lipoprotein cholesterol as calculated using the Friedewald  
33  
34 223 Equation, fasting triglycerides, high-density lipoprotein cholesterol; (h) blood pressure; (i)  
35  
36 224 adipocytokine (adiponectin) and inflammatory marker (high-sensitivity C-reactive protein  
37  
38 225 (hs-CRP)); (j) subcutaneous fat area and total fat area; (k) respiratory quotient, basal  
39  
40 226 metabolism, whole body dual-energy x-ray absorption (DXA), eating behavior questionnaire,  
41  
42 227 and calorie and glucose intake; (l) area of abdominal muscle as measured via CT; and (m) bone  
43  
44 228 density in the fourth lumbar vertebra as measured via CT. Levels of BAP, TRACP5b, insulin,  
45  
46 229 adipocytokine (adiponectin), inflammatory markers (hs-CRP), and  $\alpha$ 1-microglobulin will be  
47  
48 230 measured at a central laboratory (LSI Medience Corporation, Tokyo, Japan).

49  
50 231 Total body composition will be determined by whole body DXA using a fanbeam bone  
51  
52 232 densitometer (Discovery™ DXA system; Hologic, Inc., Marlborough, MA, USA), and all the  
53  
54 233 scans will be analyzed using Discovery™ software version 13.3.0.1 (Hologic, Inc.,  
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56 234 Marlborough, MA, USA), which contains the Hologic Advanced Body Composition™

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5 235 assessment and InnerCore™ visceral adipose tissue assessment. Two certified technologists  
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7 236 perform all scans.  
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9 237 Serious adverse events (AEs) will be documented and reported per regulatory  
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11 238 requirements.  
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15 240 **Data collection**

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17 241 **Study visits and examinations**

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19 242 The schedule for the study visits and data collection is summarized in Table 1.  
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256 **Table 1.** Schedule of data collection

	Before observation period	Start of administration	Dosing period				
			2 weeks	4 weeks	8 weeks	12 weeks	24 weeks
Allowance	Within 4 weeks	Day 0	Within $\pm 1$ week	Within $\pm 1$ week	Within $\pm 2$ weeks	Within $\pm 2$ weeks	Within $\pm 4$ weeks
Visit	1	2	3	4	5	6	7
Informed consent	X						
Patient characteristics	X						
Study drug administration		←———— X —————→					
Symptom check		←———— X —————→					
Adverse events check		←———— X —————→					
Visceral fat area measured via CT		X					X
Body weight	X	X	X	X	X	X	X
Heart rate, blood pressure		X	X	X	X	X	X
Blood tests		X	X	X	X	X	X
Blood chemistry		X	X	X	X	X	X
Urine tests		X	X	X	X	X	X
Insulin, bone marker, inflammation marker		X				X	X
$\alpha 1$ -microglobulin		X					X
Adipocytokine		X					X
Waist circumference	X	X	X	X	X	X	X
Hand griping test		X				X	X
Medication adherence check			X	X	X	X	X
Special examination *		X					X
Screening blood examination	X						

257 \*: Special examination includes whole body DXA, dietary behavior questionnaire, respiratory  
 258 quotient, basal metabolism, and calorie and glucose intake for patients.



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5 259 **CT, computed tomography; DXA, dual-energy x-ray absorption.**  
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9 261 **Data management, monitoring, and auditing**

10 262 The investigators (or their delegates) will maintain individual records for each patient as source  
11 263 data, which will include a log of informed consent, medical records, laboratory data, and other  
12 264 records or notes, as appropriate. All entries in the case report form (CRF) must be backed up by  
13 265 the relevant source data. CRFs must be completed in a timely manner.

14 266 All data will be collected by the independent data management center. There will be no  
15 267 direct communication between investigators and the coordinating data center. The clinical data  
16 268 entry (double data entry), coding, data management, and reporting will be performed using the  
17 269 data management system ACRess (Fujitsu, Tokyo, Japan).

18 270 A monitor will confirm that the investigational team is adhering to the protocol, data are  
19 271 being accurately recorded in CRFs, AEs have been properly documented on CRFs, severe AEs  
20 272 (SAEs) have been forwarded to the coordinating investigator and the provider of the  
21 273 investigational product, and the SAEs that met the criteria for reporting have been forwarded to  
22 274 the Institutional Review Board (IRB). An interim analysis will not be performed.

23 275 The study may be audited or inspected by a third party (Increase Co., Ltd.; Tokyo,  
24 276 Japan). In case of an audit, the investigators must provide study documentation to the auditor. If  
25 277 an audit or inspection occurs, the investigators at the study site must discuss the findings and  
26 278 any relevant issues.

27 279

28 280 **AEs**

29 281 Investigators must record all AEs in the patients' CRFs. All AEs are to be followed up  
30 282 continually during their course until the end of the trial. All SAEs must be reported to all  
31 283 investigators and discussed.

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5 285 **Statistical analysis**  
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7 286 The analyses of the primary and secondary efficacy endpoints will be performed using the full  
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9 287 analysis set. Safety analysis will be conducted in the safety analysis population. For the baseline  
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11 288 variables, summary statistics will be constructed using frequencies and proportions for  
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13 289 categorical data, and means and SDs for continuous variables. Patient characteristics will be  
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15 290 compared using Pearson's chi-square test or Fisher's exact test for categorical outcomes, and  
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17 291 Student's t-test for continuous variables, as appropriate.  
18

19 292 For the primary analysis to evaluate treatment efficacy, the least square mean  
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21 293 difference in the rate of visceral fat reduction between ipragliflozin and metformin treatment at  
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23 294 week 24 and its 95% confidential interval (CI) will be estimated using an analysis of covariance  
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25 295 model adjusted for allocation factors (i.e., age, HbA1c, and abdominal circumference). As a  
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27 296 sensitivity analysis, a mixed-effects model for repeated measures, the last observational carried  
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29 297 forward method, and the multiple imputation method will be applied to examine the effect of  
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31 298 missing data. The secondary analysis will be performed in the same manner as the primary  
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33 299 analysis. Data regarding hypoglycemia, dehydration, urinary tract infection, drug eruption, and  
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35 300 other AEs will be evaluated during the safety analysis. The frequencies of AEs will be compared  
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37 301 using Fisher's exact test. A sub-group analysis based on patient characteristics (i.e., diabetes  
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39 302 duration, drug combinations, age, and BMI) will be performed to investigate mechanisms  
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41 303 underlying patient responses to ipragliflozin.  
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43 304 All comparisons have been planned, and all p-values will be two-sided. P-values <0.05  
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45 305 will be considered statistically significant. All statistical analyses will be performed using SAS  
46  
47 306 Version 9.4 (SAS Institute, Cary, North Carolina, USA). This plan for statistical analysis was  
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49 307 developed by the chief investigator and statisticians at Chiba University, and will be finalized  
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51 308 prior to database lock.  
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56 310 **ETHICS AND DISSEMINATION**  
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5 311 **Research ethics approval and protocol amendments**

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7 312 The study protocol was approved by the following IRBs: Institutional Review Board of Chiba  
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9 313 University Hospital (ID number: G26009), Asahi General Hospital Ethics Review Committee  
10  
11 314 (ID number: 2014091602), National Hospital Organization Chiba Medical Center Research  
12  
13 315 Review Board, Seirei Sakura Citizen Hospital Ethics Committee, Chiba Rosai Hospital Ethics  
14  
15 316 Committee (ID number: 26-21), Toho University Sakura Medical Center Ethics Committee (ID  
16  
17 317 number: 2014-077), Tokyo Women's Medical University Yachiyo Medical Center Ethics  
18  
19 318 Committee (ID number: 150303), Chiba Aoba Municipal Hospital Ethics Review Committee,  
20  
21 319 Kimitsu Chuo Hospital Ethics Committee, Funabashi Central Hospital Ethics Committee (ID  
22  
23 320 number: H27-1), and Chiba Kaihin Municipal Hospital Ethics Review Committee. Other  
24  
25 321 facilities were judged at the Institutional Review Board of Chiba University Hospital, which was  
26  
27 322 the centralized IRB. Substantial amendments of the study protocol must be approved by the  
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29 323 IRBs. The study was registered in the UMIN Clinical Trials Registry (UMIN000015170).

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34 325 **Informed consent**

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36 326 All participants will receive adequate information about the nature, purpose, possible risks and  
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38 327 benefits of the trial, and alternative therapeutic choices using an informed consent protocol  
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40 328 approved by the IRB. All participants must be given ample time and opportunity to ask  
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42 329 questions and consider participation in the trial. A completed informed consent form is required  
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44 330 for enrolment in the trial. The investigators must maintain the original signed consent form, as  
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46 331 well as an additional copy of this form.

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48 332 If the blood and/or the urine specimens to be stored are to be used for another research  
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50 333 in the future, a new research plan should be prepared and sent to IRB for approval prior to study  
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52 334 commencement. Samples will be discarded anonymously.

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56 336 **Confidentiality**

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5 337 To ensure confidentiality, trial participants will be allocated a unique trial identification number  
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7 338 for use throughout the trial.  
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#### 10 340 **Dissemination**

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12 341 The findings of this trial will be disseminated via peer-reviewed publications and conference  
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14 342 presentations, and will also be disseminated to participants. **The principal investigator and other**  
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16 343 **investigators will publish the results of the clinical study.**  
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#### 20 345 **DISCUSSION**

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22 346 In this study, we evaluated the safety and efficacy of 24 weeks of treatment with either an  
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24 347 SGLT-2 inhibitor (ipragliflozin) or metformin for reducing visceral fat and plasma glucose  
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26 348 levels in Asian patients with type 2 diabetes (BMI >22 kg/m<sup>2</sup>) undergoing treatment with the  
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28 349 DPP-4 inhibitor, sitagliptin, (50 mg daily) for poor glycemic control. We also evaluated the  
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30 350 effects of each treatment on other metabolic parameters. Studies regarding the effects of SGLT-2  
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32 351 inhibitors and metformin on visceral fat reduction in patients with type 2 diabetes receiving  
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34 352 first-line treatment with DPP-4 inhibitors is limited; therefore, the design of the present study  
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36 353 provides a unique opportunity to examine alternative treatment strategies in an Asian  
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38 354 population. **Another strength of this study is the blind measurement of visceral fat by CT.**  
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41 355 Clinicians must remain conscious about weight gain following increases in insulin  
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43 356 secretion when treating patients with type 2 diabetes. Metformin allows for reductions in plasma  
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45 357 glucose levels without affecting insulin secretion by pancreatic beta cells. In addition to  
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47 358 promoting uptake in peripheral tissues (mainly muscle) and improving insulin sensitivity,  
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49 359 metformin is associated with a low risk of body weight gain. Previous researchers have further  
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51 360 revealed that combined treatment with metformin and a DPP-4 inhibitor leads to significant  
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53 361 reductions in body weight.[17] Therefore, metformin may be an effective second-line treatment  
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55 362 option in patients with symptoms refractory to treatment with DPP-4 inhibitors. However,  
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5 363 metformin has been known to induce gastrointestinal disturbances and severe lactic acidosis in  
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7 364 some patients. Furthermore, the need to take medication 2 to 3 times per day often results in  
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9 365 poor medication adherence.

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11 366 Similarly, SGLT-2 inhibitors do not affect insulin secretion. SGLT-2 inhibitors act to  
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13 367 reduce glucose reabsorption in the kidneys, thereby preventing increases in blood glucose  
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15 368 levels, reducing the burden of pancreatic beta cells, restoring insulin secretion, and improving  
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17 369 glucose toxicity and insulin resistance.[8] In clinical studies, it has been reported that treatment  
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19 370 with SGLT-2 is associated with improvements in insulin sensitivity [18] and reductions in body  
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21 371 weight.[19, 20] In one clinical study, combined treatment with dapagliflozin and metformin  
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23 372 produced significant reductions in visceral fat.[10] These findings indicate that such treatment  
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25 373 may aid in lowering the risk of several conditions associated with high levels of visceral fat,  
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27 374 such as arteriosclerosis. Furthermore, once-daily drug administration is sufficient, which may  
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29 375 increase medication adherence.

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31 376 In the Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes  
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33 377 (EMPA-REG OUTCOME) study, empagliflozin exerts cardioprotective effects.[11] Therefore,  
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35 378 our findings may provide further evidence regarding the cardioprotective effects of SGLT-2  
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37 379 inhibitors.

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39 380 Further studies have revealed that adjunct treatment with dapagliflozin in patients with  
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41 381 symptoms refractory to DPP-4 inhibitor treatment resulted in HbA1c reductions of 0.5% and  
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43 382 body weight reductions of 2.1 kg.[21] In a comparative study of a single treatment with either  
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45 383 empagliflozin or metformin, metformin treatment resulted in HbA1c reductions of 0.56% and  
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47 384 body weight reductions of 1.3 kg at 90 weeks after administration, while empagliflozin  
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49 385 treatment resulted in HbA1c reductions of 0.63% and body weight reductions of 4.0 kg.[15] In a  
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51 386 comparative study of single treatment with either ipragliflozin (50 mg) or metformin (up to  
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53 387 1500 mg), no significant differences in HbA1c were observed at 12 weeks, although  
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55 388 ipragliflozin treatment resulted in body weight reductions of 0.78 kg.[22] Based on these

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5 389 previous findings, we speculate that combined treatment with an SGLT-2 inhibitor and a DPP-4  
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7 390 inhibitor results in comparable reductions in blood glucose level and a greater visceral fat  
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9 391 reduction than combined treatment with metformin and a DPP-4 inhibitor. Previous researchers  
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11 392 have provided a strong rationale for dual therapy with a DPP-4 inhibitor and an SGLT-2  
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13 393 inhibitor.[23] This study aims to provide new insight on the most appropriate combination of  
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15 394 DPP-4 and SGLT-2 inhibitors, which may lead to the development of new treatment options for  
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17 395 patients with type 2 diabetes.

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19 396 Although reductions in visceral fat are important for reducing the impact of metabolic  
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21 397 disorders and preventing complications, such as atherosclerosis, there are currently no studies  
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23 398 that have been conducted to compare the effects of SGLT-2 inhibitors and metformin on visceral  
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25 399 fat reduction in patients taking DPP-4 inhibitors. The administration of SGLT-2 inhibitors,  
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27 400 particularly in patients with poor glycemic control despite treatment with DPP-4 inhibitors, may  
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29 401 exert such beneficial effects. However, the risk of sarcopenia and osteopenia remains a concern.  
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31 402 Therefore, it is necessary to clarify specific changes in body composition, rather than reductions  
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33 403 in body weight alone, to evaluate such risks. Our study will provide evidence regarding the  
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35 404 safety and efficacy of the SGLT-2 inhibitor ipragliflozin as a second-line treatment for reducing  
36  
37 405 visceral fat and blood glucose levels in patients with type 2 diabetes.

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1  
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4  
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7 416

8  
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10  
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12  
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14  
15 420 **role in the design of this study and will not have any role during its execution, analyses,**  
16  
17 421 **interpretation of the data, or decision to submit results.**  
18

19 422

20  
21 423 **Competing interests**

22  
23 424 KY received research grants from Astellas Pharma Inc. and MSD K.K. received a lecture fee  
24  
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26  
27 426 interest are declared for other authors.  
28

29 427

30  
31 428 **Ethics approval**

32  
33 429 The protocol was approved by the Institutional Review Board of each participating hospital.  
34

35 430

36  
37 431 **Author Contributions**

38  
39 432 All authors made a significant contribution to the conception and design of the study protocol.  
40  
41 433 YK designed the original concept. The protocol was written by MK, KI, TI, KK, and MT, and it  
42  
43 434 was critically reviewed by TH, RS, ST, KN, YS, IT, TT, NH, NK, DU, and KY. All authors  
44  
45 435 provided approval for the publication of the manuscript.  
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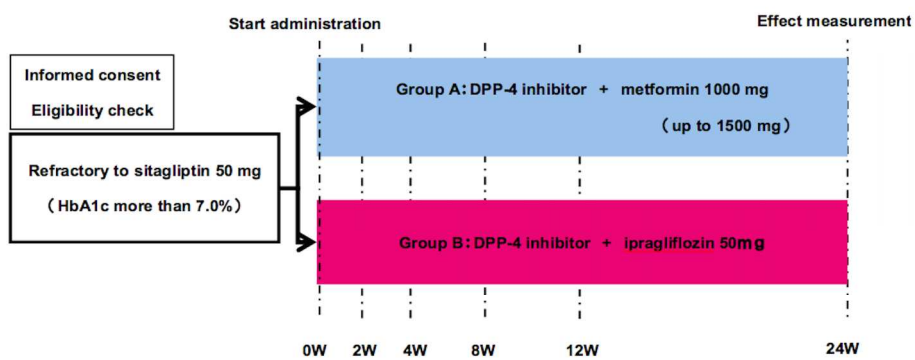
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**Figure Legends**

**Figure 1.** Schematic depiction of the trial design. Eligible participants are randomly assigned to a 24-week treatment regimen with either ipragliflozin (50 mg daily) or metformin (1000 mg daily, up to 1500 mg). HbA1c, glycosylated hemoglobin; DPP-4, dipeptidyl peptidase-4.

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**SUPPLEMENTARY APPENDIX 1****Collaborators**

PRIME-V study group

Asahi General Hospital: Hidetaka Yoko, Shunichiro Onishi and Kazuki Kobayashi

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Tetsuya Okazaki, Kenchi Yui and Emi Ohara

Kujyukuri Home Hospital: Kou Ishikawa

Kouyukai Memorial Hospital: Akiko Hattori and Masaya Yamaga

Sannou Hospital: Ryouta Shimousa

Seirei Sakura Citizen Hospital: Kana Ide, Mayumi Shoji and Ryouichi Ishibashi

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6 Sousa Citizen Hospital: Yusuke Baba, Masaya Yamaga and Ryoichi Ishibashi  
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8 Tamura Memorial Hospital: Kenichi Sakamoto and Shintaro Ide  
9

10 Toho University Sakura Medical Center: Ichiro Tatsuno, Atsuto Saiki and Yasuhiro Watanabe  
11

12 Tokuyama Clinic: Takahiko Tokuyama  
13

14 Tokyo Women's Medical University Yachiyo Medical Center: Jun Ogino, Naotake Hashimoto,  
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16 Chihiro Yoneda and Kana Tajima  
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**WHO Trial Registration Data Set**

<b>DATA CATEGORY</b>	<b>INFORMATION</b>
Primary registry and trial identifying number	UMIN000015170
Date of registration in primary registry	21 September, 2014
Secondary identifying numbers	Institutional Review Board of Chiba University approved number: G26009
Source(s) of monetary or material support	Chiba University
Primary sponsor	Chiba University  1-8-1 Inohana, Chuo-ku, Chiba-shi, Chiba 260-8677, Japan  +81-43-222-7171
Secondary sponsor(s)	Astellas Pharma Inc.  2-5-1, Nihonbashi-Honcho, Chuo-Ku, Tokyo 103-8411, Japan  +81-3-3244-3000
Contact for public queries	Masaya Koshizaka, MD, PhD [+81-43-222-7171] [overslope@chiba-u.jp]  Ko Ishikawa, MD, PhD [+81-43-222-7171] [ishikawako@chiba-u.jp]  Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine, Japan, 1-8-1 Inohana, Chuo-ku, Chiba-shi, Chiba 260-8677, Japan

<b>DATA CATEGORY</b>	<b>INFORMATION</b>
Contact for scientific queries	Koutaro Yokote, MD, PhD [+81-43-226-2092][kyokote@faculty.chiba-u.jp] Professor, Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine, Japan, 1-8-1 Inohana, Chuo-ku, Chiba-shi, Chiba 260-8677, Japan
Public title	Prospective and randomized controlled study on the efficacy and safety of ipragliflozin and metformin for visceral fat reduction in patients being treated with dipeptidyl peptidase-4 (DPP-4) inhibitors for poor glycemic controlled type-2 diabetes (PRIME-V)
Scientific title	Prospective and randomized controlled study on the efficacy and safety of ipragliflozin and metformin for visceral fat reduction in patients being treated with DPP-4 inhibitors for poor glycemic controlled type-2 diabetes (PRIME-V)
Countries of recruitment	Japan
Health condition(s) or problem(s) studied	Type 2 diabetes mellitus
Intervention(s)	Treatment group: DPP-4 inhibitor sitagliptin 50 mg, ipragliflozin 50 mg Control group: DPP-4 inhibitor sitagliptin 50 mg, metformin 1000 mg (can be increased up to 1500 mg after 12 weeks from the initial 500 mg)
Key inclusion and exclusion criteria	<b>Inclusion criteria</b> Eligible patients are those who meet the following inclusion criteria: (a) diagnosis of type 2 diabetes, confirmed in accordance with Japanese guidelines[16]; (b) age between 20 and 75 years; (c) inadequate control of plasma glucose levels despite treatment with 50 mg of the DPP-4 inhibitor sitagliptin for >12 weeks; (d) glycosylated hemoglobin (HbA1c, which provides an indication of the average blood glucose concentration of a patient over the previous 3 months) level >7.0% or <10.0% (according to the National Glycohemoglobin Standardization Program [NGSP]); (e) body mass index (BMI) >22 kg/m <sup>2</sup> ; (f) estimated glomerular filtration rate >50 mL/min/1.73 m <sup>2</sup> ; and (g) an adequate



DATA CATEGORY	INFORMATION
	<p>understanding of the contents of the trial and provision of written informed consent.</p> <p>Exclusion criteria</p> <p>Patients meeting any of the following criteria will be excluded from the trial: (a) diagnosis of type 1 diabetes; (b) history of metabolic acidosis, diabetic coma, and/or pre-coma up to 6 months prior to providing consent; (c) history of serious infections requiring insulin treatment, prior/upcoming surgeries, and/or severe injuries; (d) considerable loss of kidney function (blood creatinine level &gt;1.3 mg/dL in men or &gt;1.2 mg/dL in women) and/or need for dialysis (including peritoneal dialysis); (e) serious liver damage; (f) history of stroke, myocardial infarction, heart failure, or other severe cardiovascular complications requiring hospitalization; (g) use of oral hypoglycemic agents other than DPP-4 inhibitors at the start of the trial; (h) pregnancy, nursing, or plans to become pregnant; (i) history of chemical sensitivity to DPP-4 inhibitors, Sodium-dependent glucose transporter-2 inhibitors, and/or metformin; (j) current diagnosis of, or at risk for, urinary tract infection and/or dehydration; (k) positive for ketone bodies; (l) history of lactic acidosis; (m) excessive alcohol consumption; (n) history of bone fracture caused by osteoporosis; (o) need for computed tomography (CT) scan within 3 months prior to providing written consent; and/or (p) determination of ineligibility by the attending physician for any other reason.</p>
Study type	<p>Interventional</p> <p>Allocation: randomized</p> <p>Intervention model: parallel assignment by computer program</p> <p>Masking: blind (outcomes assessor)</p> <p>Primary purpose: reductions in visceral fat</p> <p>Phase IV</p>
Date of first enrolment	January 2015
Target sample size	106
Recruitment status	No longer recruiting

<b>DATA CATEGORY</b>	<b>INFORMATION</b>
Primary outcome(s)	The rate of change in the total area of visceral fat in each group, as measured via CT following the 24-week treatment period
Key secondary outcomes	(a) HbA1c (NGSP); (b) body weight and BMI; (c) waist circumference; (d) bone markers (alkaline phosphatase, bone alkaline phosphatase, and tartrate-resistant acid phosphatase-5b; (e) muscle strength; (f) fasting plasma glucose level, homeostatic model assessment (HOMA)-b, and HOMA-R; (g) cholesterol level (total cholesterol, low-density lipoprotein cholesterol as calculated using the Friedewald Equation, fasting triglycerides, and high-density lipoprotein cholesterol); (h) blood pressure; (i) adipocytokine (adiponectin) and inflammatory markers; (j) subcutaneous fat area and total fat area; (k) respiratory quotient, basal metabolism, whole body dual-energy x-ray absorption, eating behavior questionnaire, and calorie and glucose intake; (l) area of abdominal muscle as measured via CT; and (m) bone density in the fourth lumbar vertebra as measured via CT.

**Protocol Version and Amendment Tracking**

<b>Version Number/Amendment</b>	<b>Date</b>
1.0	27/May/2014
1.1 Revision	30/June/2014
1.2 Revision	8/September/2014
1.3 Revision	1/December/2014
1.4 Revision	6/February/2015
1.5 Revision	25/March/2015
1.6 Revision	19/May/2015
1.7 Revision	7/September/2015
1.8 Revision	24/November/2015
1.9 Revision	1/March/2016
2.0 Revision	21/September/2016

## Research Organization

### 1) Principal Investigator

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Yachiyo Medical Center

Nobuichi Kuribayashi, MD, PhD

Misaki Naika Clinic

Daigaku Uchida, MD, PhD

Hotaruno Central Naika

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Department of Radiology, Sannou Hospital

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Kazuki Kobayashi, MD, PhD

Department of Diabetes/Metabolism, Asahi General Hospital

Ko Ishikawa, MD, PhD

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28 **4) Study Coordinating Management Committee**

29 Koutaro Yokote, MD, PhD

30 Professor, Department of Clinical Cell Biology and Medicine, Chiba University Graduate  
31 School of Medicine

32 Professor, Department of Medicine, Division of Diabetes, Metabolism and Endocrinology,  
33 Chiba University Hospital  
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36 Hideki Hanaoka

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40 Medicine  
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43 Takatoshi Sato

44 Clinical Research Center, Chiba University Hospital  
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49 **5) Study Coordinating Management Office**

50 Clinical Research Center, Chiba University Hospital  
51  
52

53 **6) Auditors**

54 Increase Co., Ltd. (Tokyo, Japan)  
55  
56  
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58 **7) Patient Registration Center / Allocation / Data Management**

59 Chiba University Clinical Trial Data Center  
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6 Mayumi Negishi, Mayumi Matsui and Mayumi Ogawa

7 The clinical data entry (double data entry), coding, data management, the allocation sequence  
8 generation, and reporting will be performed using the data management system ACRess  
9 (Fujitsu, Tokyo, Japan).  
10  
11

## 12 13 **8) Statistical Analysis**

14 Sho Takahashi

15 Clinical Research Center, Chiba University Hospital

16 Kengo Nagashima and Yasunori Sato

17 Department of Global Clinical Research / Biostatistics, Chiba University, Graduate School of  
18 Medicine  
19

## 20 21 22 **9) Independent Data Monitoring Committee**

23 Shunsuke Furuta, MD, PhD

24 Department of Allergy and Collagen Disease, Chiba University Hospital

25 Kaori Tachibana, MD, PhD

26 Department of Diabetes/Metabolic Endocrinology, Japanese Red Cross Narita Hospital

27 Tsuyoshi Matsumoto, MD, PhD

28 Department of Diabetes/Metabolism, Funabashi Central Hospital  
29

## 30 31 32 **10) Project Support Organizations**

33 Central Laboratory: LSI Medience Corporation (Tokyo, Japan)

34 Image processing Contact Research Organization (CRO): Micron Inc. (Tokyo, Japan)  
35

## 36 37 38 **11) Monitoring**

39 Increase Co., Ltd. (Tokyo, Japan)  
40

## 41 42 43 **12) Other**

44 To conduct this study, an agreement was signed between Chiba University and Astellas Pharma  
45 Inc. (Tokyo, Japan). Astellas Pharma Inc. funds this study.  
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 SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*
 

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Section/item	Item No	Description	Addressed on page number
<b>Administrative information</b>			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1 _____
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	4, 16 _____
	2b	All items from the World Health Organization Trial Registration Data Set	Appendix 3-6 _____
Protocol version	3	Date and version identifier	Appendix 7 _____
Funding	4	Sources and types of financial, material, and other support	20 _____
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 2, 20 _____
	5b	Name and contact information for the trial sponsor	20, Appendix 3 _____
	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	20 _____
	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)	Appendix 8-10 _____

1 **Introduction**

2

3 Background and 6a Description of research question and justification for undertaking the trial, including summary of relevant 5-6  
 4 rationale studies (published and unpublished) examining benefits and harms for each intervention  
 5  
 6 6b Explanation for choice of comparators 5  
 7  
 8 Objectives 7 Specific objectives or hypotheses 6  
 9  
 10 Trial design 8 Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group),  
 11 allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) 6, 9  
 12  
 13

14 **Methods: Participants, interventions, and outcomes**

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16 Study setting 9 Description of study settings (eg, community clinic, academic hospital) and list of countries where data will  
 17 be collected. Reference to where list of study sites can be obtained 9, Appendix 1-2  
 18  
 19 Eligibility criteria 10 Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and  
 20 individuals who will perform the interventions (eg, surgeons, psychotherapists) 8  
 21  
 22 Interventions 11a Interventions for each group with sufficient detail to allow replication, including how and when they will be  
 23 administered 10  
 24  
 25 11b Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose  
 26 change in response to harms, participant request, or improving/worsening disease) 10  
 27  
 28 11c Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence  
 29 (eg, drug tablet return, laboratory tests) 10  
 30  
 31 11d Relevant concomitant care and interventions that are permitted or prohibited during the trial 10-11  
 32  
 33 Outcomes 12 Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood  
 34 pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg,  
 35 median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen  
 36 efficacy and harm outcomes is strongly recommended 9-12  
 37  
 38 Participant timeline 13 Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for  
 39 participants. A schematic diagram is highly recommended (see Figure) 12-14  
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1	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	7
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4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	9
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7	<b>Methods: Assignment of interventions (for controlled trials)</b>			
8	Allocation:			
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11	Sequence	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
12	generation			
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17	Allocation	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
18	concealment			
19	mechanism			
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21	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
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24	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
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28		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	10
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32	<b>Methods: Data collection, management, and analysis</b>			
33				
34	Data collection	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12-14
35	methods			
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40		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	14
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1	Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	14
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5	Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	15
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8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	15
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10		20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	15
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15	<b>Methods: Monitoring</b>			
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17	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	14
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22		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	14
23				
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26	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	14
27				
28				
29	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	14
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33	<b>Ethics and dissemination</b>			
34				
35	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	16
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38	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	16
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1	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	<u>16</u>
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4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	<u>16</u>
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7	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	<u>16-17</u>
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10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	<u>20</u>
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14	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	<u>7</u>
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17	Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	<u>14</u>
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20	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	<u>17</u>
21				
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25		31b	Authorship eligibility guidelines and any intended use of professional writers	<u>17</u>
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27		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	<u>Not applicable</u>
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30	<b>Appendices</b>			
31				
32	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	<u>16, Consent form</u>
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35	Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	<u>Not applicable</u>
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\*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.