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## Diabetes Prevention with active Vitamin D (DPVD study): a protocol for a randomized, double-blind, placebo- controlled study

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**Diabetes Prevention with active Vitamin D (DPVD study):  
a protocol for a randomized, double-blind, placebo-controlled study**

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**Strengths and limitations of this study**

- This study will elucidate the effect of eldecacitol, active vitamin D, to prevent the incidence of type 2 diabetes in individuals with impaired glucose tolerance.
- A randomized, double-blinded, placebo-controlled design.
- Because, in this study, the study participants are at high risk for diabetes, all participants visit clinics to be followed by physicians every 3 months, and they are prescribed active vitamin D formulations at that visit, the drug compliance and the follow-up ratio should be better than those in other trials where study participants, almost healthy people, receive vitamin D supplements.
- Because this study consists of only Japanese participants, it is unknown whether the results of this study are adapted to other ethnicity.

## Abstract

**Introduction:** Recent research suggests that vitamin D deficiency may cause not only bone diseases but also a range of non-skeletal disease. However, most of these data come from observational studies, and clinical trial data of the effects of vitamin D supplementation on individuals with prediabetes are scarce and inconsistent. The aim of Diabetes Prevention with active Vitamin D (DPVD) study is to assess the effect of eldcalcitol, active vitamin D analog, on the incidence of type 2 diabetes among individuals with prediabetes.

**Methods and analysis:** DPVD is an on-going prospective, multicenter, randomized, double-blind outcome study in individuals with impaired glucose tolerance. Participants, men and women aged  $\geq$  30 years, are randomized to receive eldcalcitol or placebo. They are also given a brief (5 to 10 minutes long) talk about appropriate calorie intake from diet and exercise at each 12-week visit. The primary endpoint is the accumulate incidence of type 2 diabetes. Secondary endpoints include the improving ratio from IGT to normoglycemia at 48, 96, and 144 weeks. Follow-up is estimated to span 144 weeks.

**Ethics and dissemination:** All protocols and the informed consent form comply with the Ethics Guideline for Clinical Research (Japan Ministry of Health, Labor and Welfare). The study protocols is approved by the Institution Review Board at Kokura Medical Association and University of Occupational and Environmental Health. The study will be implemented in line with the CONSORT statement.

**Trial registration number:** UMIN Clinical Trials Registry: UMIN000010758

**INTRODUCTION**

Type 2 diabetes is an important risk factor for cardiovascular disease and causes a variety of complications. Moreover, the incidence of type 2 diabetes is currently increasing and has become a major burden to healthcare systems and societies worldwide [1]. In Japan, there are approximately 9.5 million persons with people with diabetes and 109,000 persons per year are diagnosed with diabetes [2]. Additionally, 552 million people worldwide will have type 2 diabetes by the year 2030 [3]. The incidence of type 2 diabetes from prediabetes [impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT)] is about 10% (5.8–13.2%) per year [4-7]. While diabetes is an irreversible state, prediabetes can be reverted to a normal glucose state. Therefore, many clinical trials have aimed to evaluate the reduction of the incidence of type 2 diabetes by targeting individuals with prediabetes.

Active vitamin D is currently prescribed as the drug of choice for osteoporosis and hypocalcemia, but it was reported that vitamin D and active vitamin D have additional metabolic effects aside from that on bone and calcium metabolism [8-10] because vitamin D receptors have been found in various tissues, brain, pancreas, breast, kidney, colon, prostate, and immune cells [11-14]. One of the additional effects of vitamin D is that exerted on the glucose metabolism. Many observational studies suggested that the serum 25-hydroxy vitamin D<sub>3</sub> [25(OH)D<sub>3</sub>] level is inversely associated with the incidence of type 2 diabetes [15-19]. Thus, some small-scale clinical trials have evaluated whether vitamin D and active vitamin D have the effect of improving insulin resistance and glucose metabolism, but the results are still controversial [20-26].

With the aim of further addressing this issue, we designed a randomized controlled trial to evaluate whether active vitamin D, eldecaltitol, can prevent the incidence of type 2 diabetes among individuals with IGT and whether it can normalize blood glucose levels.

**Objectives and endpoints**

The objective of the present study is to elucidate the therapeutic effect of eldecaltitol (active vitamin D) on the prevention of type 2 diabetes among individuals with IGT. The primary endpoint is the hazard ratio of type 2 diabetes onset during 144 weeks of the study period. The secondary endpoints

include that the improving ratio from IGT to normoglycemia at 48, 96, and 144 weeks.

## METHODS AND ANALYSES

### Study design

This Diabetes Prevention with active Vitamin D (DPVD) study is designed as a prospective, multicenter, randomized, double-blind, parallel group comparison study. In total, 750 adults with IGT will be randomly assigned to either the active vitamin D or control group, and treated until the onset of type 2 diabetes or the end of treatment period (144 weeks).

### Participant eligibility

Individuals with IGT who meet all inclusion criteria and none of the exclusion criteria (Table 1) will be enrolled.

### Intervention

After confirming that an individual conforms to the inclusion and not to the exclusion criteria, a sub-investigator obtains written informed consent from him/her. The sub-investigator fills out the participants' recognition code list with the date of acquisition of the informed consent, the participant registration number (PRN), and personal information necessary for identifying the PRN with the participant. The PRN is composed of name of the institution, sex (male [M] or female [F]), age (30–54 years [Y];  $\geq 55$  years [E]), 75-g OGTT 2h-PG ( $< 170$  mg/dl [L];  $\geq 170$  mg/dl  $\leq$  [H]), and the number of visits. For example, in a hospital, a male participant, aged 43 years, with 176 mg/dl of 2h-PG, who was the fifth participant recruited in this study would have a PRN of ○○ hospital MYH5. Randomization list is made by a responsible person in the assignment center at Kokura Medical Association with sex, age, and 75-g OGTT 2h-PG as stratification factors using a permuted block procedure prior to the first patient entry. The list will be kept in a safe in the assignment center until it is time to open the safe. The location of the key will not be disclosed to the investigators or sub-investigators. The key will be retrieved only after the trial is finished and data are fixed, except in

the case of emergencies. After confirming eligibility, a staff member in the assignment center allocates a participant to either the active vitamin D group or the control group using central randomization method. Active vitamin D group: Participants receive 0.75 µg of eldecalcitol daily for 144 weeks (3 years) or until the incidence of diabetes. Eldecalcitol is an active vitamin D<sub>3</sub>, which is added with the hydroxypropyloxy group to the position of 2β in calcitriol [1,25(OH)<sub>2</sub>D<sub>3</sub>], leading to an enhanced calcium absorption from the intestine. Control group: Participants receive one capsule of placebo daily for 144 weeks (3 years) or until the incidence of diabetes. Participants in both groups are seen at 12-week intervals by their sub-investigators. A brief (5 to 10 minutes long) talk on appropriate calorie intake from diet (ideal body weight × 25–30 kcal) and exercise will be given to each participant based on the attached common sheet.

**Outline of the study (Fig. 1)**

After registration and assignment to treatments, all participants are seen at 12-week intervals by their sub-investigators. Body height, body weight and blood pressure are to be measured, blood samples are taken at baseline and every 12 weeks thereafter. A 75-g OGTT will be performed at baseline and annually. Bone mineral density (BMD) is not an obligatory measurement but will be evaluated upon request from participants. If fasting plasma glucose (FPG) ≥126 mg/dl or HbA<sub>1c</sub> ≥6.5% (≥48 mmol/mol) is identified, a 75-g OGTT will be performed within 4 weeks. When HbA<sub>1c</sub> ≥6.5% (≥48 mmol/mol) and at least one of the following is recognized, the participant is diagnosed with diabetes: FPG ≥126 mg/dl, 75-g OGTT 2h-PG ≥200 mg/dl, or casual plasma glucose (PG) ≥200 mg/dl. However, if HbA<sub>1c</sub> ≥6.5% (≥48 mmol/mol) plus FPG ≥140 mg/dl or casual PG ≥210 mg/dl are identified, individuals will expected to present hyperglycemia secondary to 75-g OGTT. Therefore, diabetes can be diagnosed without 75-g OGTT. The participants with diabetes will be excluded from the followed-up data analysis. Conversely, when the participants achieve normoglycemia, defined as meeting all three glycemic criteria [FPG <110 mg/dl, 2h-PG <140 mg/dl, and HbA<sub>1c</sub> <6.5% (<48 mmol/mol)] the date will be recorded, and they will be followed-up as analysis subjects until diabetes onset.

## Primary endpoint

The primary endpoint is the hazard ratio of type 2 diabetes onset during 144 weeks of the study period: Active vitamin D group vs. Control group. We consider that the comparison of the treatment effect on type 2 diabetes onset between the two groups is the best measurement to evaluate the improvement of insulin resistance and glucose tolerance.

## Secondary endpoints

- 1) Improving ratio from IGT to normoglycemia at 48, 96, and 144 weeks.
- 2) Hazard ratios and 95% confidence intervals (CI) of type 2 diabetes onset in each subgroup at baseline: age ( $\geq$  /  $<65$  years), sex (men/women), presence or absence of hypertension (systolic  $\geq 140$  mmHg and/or diastolic  $\geq 90$  mmHg), dyslipidemia (LDL-cholesterol  $\geq 140$  g/dl and/or triglycerides  $\geq 150$  mg/dl), obesity (body mass index  $\geq$  /  $<25$  kg/m<sup>2</sup>), family history of diabetes, FPG ( $\geq$  /  $<110$  mg/dl), 2h-PG ( $\geq$  /  $<170$  mg/dl), 25(OH)D<sub>3</sub> ( $\geq$  /  $<25$  ng/ml), homeostasis model assessment of insulin resistance (HOMA-R) ( $\sim 1.6$  /  $1.61-2.49$  /  $2.5\sim$ ), and insulinogenic index ( $\geq$  /  $<0.4$ ).
- 3) Hazard ratios and 95% CI of type 2 diabetes development after adjusting for confounding factors: age ( $\geq$  /  $<65$  years), sex (men/women), presence or absence of hypertension (systolic  $\geq 140$  mmHg and/or diastolic  $\geq 90$  mmHg), dyslipidemia (LDL-cholesterol  $\geq 140$  g/dl and/or triglycerides  $\geq 150$  mg/dl), obesity (body mass index  $\geq$  /  $<25$  kg/m<sup>2</sup>), family history of diabetes, FPG ( $\geq$  /  $<110$  mg/dl), 2h-PG ( $\geq$  /  $<170$  mg/dl), 25(OH)D<sub>3</sub> ( $\geq$  /  $<25$  ng/ml), HOMA-R ( $\geq$  /  $<2.5$ ), insulinogenic index ( $\geq$  /  $<0.4$ ).
- 4) Hazard ratios of the incidence of adverse events
- 5) Amount and percentage change in HOMA-R during the 144 weeks
- 6) Changes in serum levels of the receptor activator of the nuclear factor kappa B ligand (RANKL), osteoprotegerin, osteocalcin, and leptin during 48 weeks (1 year) and the association of these items with insulin resistance [HOMA-R and quantitative insulin sensitivity check index (QUICKI)]

## Statistical analyses

Intention-to-treat population comprises all participants who undergo randomization and receive at

least one dose of study drug, and who will be analyzed for the assessment of effectiveness and safety. In this analysis plan, the data set is called the full analysis set. Per protocol set, comprising all participants who undergo randomization and have no violation of inclusion and exclusion criteria, is defined as the secondary population for sensitivity analysis. In the summary of the data in each specified period, if data are obtained beyond an acceptable range of the prescribed observation date or by other than the default method or condition, the data will be treated as missing data. Basic statistics (mean, standard deviation) are calculated in each treatment arm and each time that an examination is performed for all continuous variables. If necessary, basic statistics are calculated as a whole. In FPG, HbA1c, and other laboratory data, differences between the values at baseline and those taken every 12 weeks, and their percent changes from baseline are calculated. Basic statistics, standard errors, or 95% CI are also calculated in these items. The distribution will be summarized in contingency tables for each treatment arm and each time that an examination is performed for all categorical variables. All significance tests and CI were two-sided and performed or constructed at the 5% significance level.

**Sample size**

Sample size was calculated with an 80% power (1-β), a 0.05 α error, and a two-sided test. Based on Log-rank test in Kaplan-Meier survival curve method, we assumed that the accumulating incidence of type 2 diabetes in the control group is 23.14% (8.4% annually) and that in the active vitamin D group is 14.81% (5.2% annually) during 3 years. Thus, the relative risk reduction and drop-out ratio are assumed at 36% and 7%, respectively. As a result, 375 participants are required in each group (total 750).

**Interim analysis**

According to the incidence of diabetes, it is assumed that at least 133 participants will develop diabetes during the study period. The total number of interim analyses will be two. When 1 year has passed after the registration of the last participant, the first interim analysis will be conducted. Simultaneously, the drop-out ratio of participants will be confirmed. If it is more than 3% per year, recruitment of trial participants will be conducted again. The second interim analysis will be done

when 80 participants develop diabetes, that is, 60% of the expected diabetes incidence. Regarding the primary endpoint, when the difference, determined by the O'Brien-Fleming method, between the both groups is  $< 0.0005$  (in first interim analysis),  $< 0.014$  (in the second interim analysis), or  $< 0.045$  (this is not in an interim analysis but the final analysis), early completion of the trial will be decided by the Independent Monitoring Committee.

### **Ethical considerations and dissemination**

This study is being conducted according to Good Clinical Practice and the Declaration of Helsinki [27]. The study protocol was approved by the Institutional Review Board at Kokura Medical Association and University of Occupational and Environmental Health. Written informed consent was obtained from all participants before study participation. Participants were informed of their right to withdraw from the study at any time. This study was registered at the University Hospital Medical Information Network clinical trials registry (UMIN000010758).

## **DISCUSSION**

There is increasing interest in the potential health benefits from taking vitamin D supplements and active vitamin D formulations. Recent research suggests that vitamin D deficiency may cause not only bone diseases but also a range of non-skeletal diseases, such as cardiovascular diseases, cancers (eg. colorectal, breast), type 2 diabetes, infectious diseases (eg. influenza, common cold, tuberculosis), and autoimmune diseases (eg. multiple sclerosis, type 1 diabetes) [9, 28]. However, most of these data come from observational studies, and clinical trial data on the effects of vitamin D are scarce and inconsistent [24-26]. Hence, we focused on the effectiveness of eldecalcitol, active vitamin D, on the incidence of type 2 diabetes among individuals at high risk for diabetes and designed the protocol of DPVD study. This study should provide valuable information, both on the incidence of type 2 diabetes and the improvement to the normal glucose state from IGT.

As a result of necessary compromises of study design, the DPVD study protocol has a number of strengths and weaknesses. Because this study consists of only Japanese participants, it is unknown

whether the results of this study are adapted to other ethnicity. Several large-scale clinical trials to examine the benefits of vitamin D in a number of chronic diseases has recently been started around the world: U.S., Finland, New Zealand, 8 European cities, and U.K. [28, 29]. The primary endpoints are cancer, cardiovascular disease, respiratory disease, infection, hypertension, bone fracture, cognitive function, and longevity. However, there is not the primary endpoint of diabetes. As compared with these trials in which the number of participants is 2,000 to 20,000, that in our study is 750. However, our study is not considered to be inferior to others because of the following reasons. In other trials, primary endpoints are diverse; study participants are almost healthy person; there is no regular outpatient follow-up by a physician; vitamin D supplements are delivered by a mail once a year. Whereas, in our study, there is one primary endpoint; study participants are at high risk for diabetes; all participants visit clinics to be followed by physicians every 3 months; they are prescribed active vitamin D formulations at that visit. Therefore, the drug compliance and the follow-up ratio in our study should be better than those in other trials; additionally, the sample size of our study, 750 participants, should be enough to evaluate the primary endpoint.

Although vitamin D supplements are chosen to be used in most of other trials, active vitamin D formulation is chosen in our study. Humans get vitamin D from exposure to sunlight, from their diet, and from dietary supplements. Vitamin D from skin and diet is metabolized in the liver to 25-hydroxynitamin D; 25-hydroxynitamin D is metabolized in the kidneys by the enzyme 25-hydroxynitamin D-1 $\alpha$ -hydroxylase to its active form, 1,25-dihydroxynitamin D. Vitamin D itself does not have physiological effect but 1,25-dihydroxynitamin D has that: it promotes intestinal calcium absorption in the small intestine and calcium resorption from bone by the interacting with the vitamin D receptor to enhance the expression of the epithelial calcium channel and calcium- binding protein [11]. As a result, the administration of active vitamin D rather than vitamin D leads to expression of these physiological effects more effectively and promptly in bone and calcium metabolism. Therefore, we expected that active vitamin D provides non-skeletal actions such as glucose metabolism more effectively, and chose active vitamin D formulation in our study. In conclusion, results from this study will provide a clearer picture of the risk and

benefits of eldecalfol (active vitamin D) for prevention of type 2 diabetes. It should contribute to the resolution of a debate “whether vitamin D and active vitamin D is panacea for glucose metabolism [30].”

### Current study status

The DPVD study began recruiting participants in June 2013 and is expected to be reported in 2017.

### Acknowledgements

We are grateful to all study participants and all staff of this study for recruiting the participants and contributing to the accurate recording of trial data.

### Contributors

TK, GS, and SM, FK conceived and designed the study. TK drafted the protocol of the study and organized study implementation. TK, GS, TI, SM, FK, YO, and YT refined the study protocol and study implementation. SM and FK conducted the statistical analyses. TK, GS, TI, SM and YT have drafted the manuscript. All authors have read and approved the final version of the manuscript.

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### Conflict of interest

The authors declare that they have no conflict of interest.

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## Figure legend

**Figure 1** Outline of the DPVD study design.

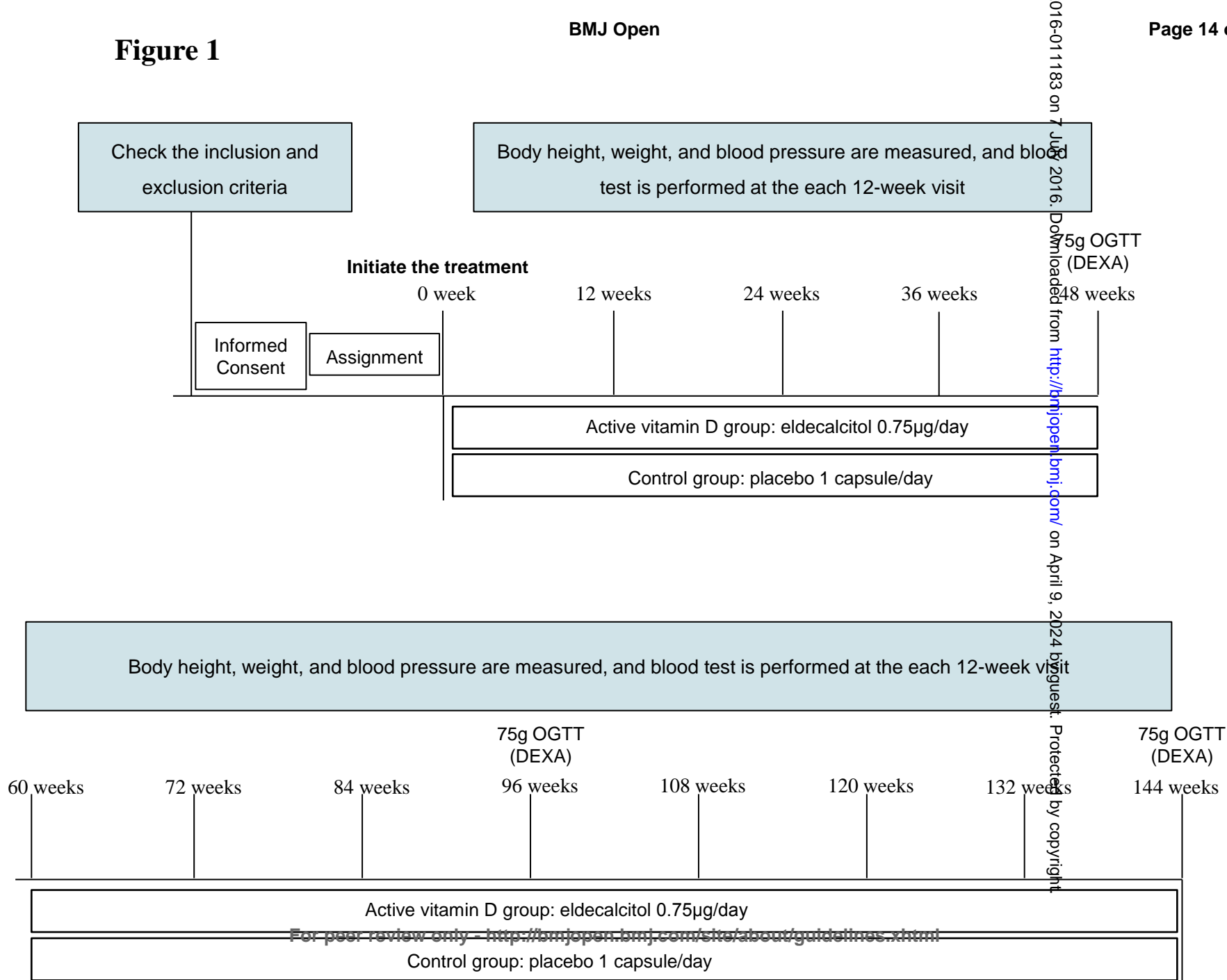
75g OGTT, 75g oral glucose tolerance test; DEXA, dual-energy X-ray absorptiometry.

**Table 1** Eligibility criteria for participation in DPVD study

Diagnostic criteria of IGT	FPG < 126 mg/dl, 75-g OGTT's 2h-PG is $140 \leq 2h-PG < 200$ mg/dl, and HbA1c < 6.5% (<48 mmol/mol). Additionally, he/she should not be medicated with any antidiabetic drug.
Inclusion criteria	Men and women aged $\geq 30$ years Individuals who are diagnosed with IGT. Serum calcium (corrected value): < 11.0 mg/dl
Exclusion criteria	Individuals who have participated in other clinical trials. Individuals who have been treated with active vitamin D, vitamin D supplement, and/or calcium preparation during last three months. Individuals who have already been diagnosed with type 1 or 2 diabetes. Individuals who have already been initiated with a drug treatment for pre-diabetes. Individuals who are pregnant or have severe diseases, such as renal insufficiency (serum creatinine > 1.5 mg/dl), hepatic insufficiency, psychosis, collagen disease, heart diseases, cerebrovascular diseases, and other conditions. A sub-investigator decides which participants' conditions are inappropriate and preclude his/her participation in the study.

IGT, impaired glucose tolerance; FPG, fasting plasma glucose; 75-g OGTT, 75-g oral glucose tolerance test; 2h-PG, 2-hour plasma glucose.

Figure 1





CONSORT 2010 checklist of information to include when reporting a randomised trial\*

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

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

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

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## Rationale and design of Diabetes Prevention with active Vitamin D (DPVD): a randomized, double-blind, placebo-controlled study

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# Rationale and design of Diabetes Prevention with active Vitamin D (DPVD): a randomized, double-blind, placebo-controlled study

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## Abstract

**Introduction:** Recent research suggests that vitamin D deficiency may cause not only bone diseases but also a range of non-skeletal diseases. However, most of these data come from observational studies, and clinical trial data of the effects of vitamin D supplementation on individuals with prediabetes are scarce and inconsistent. The aim of Diabetes Prevention with active Vitamin D (DPVD) study is to assess the effect of eldecalcitol, active vitamin D analog, on the incidence of type 2 diabetes among individuals with prediabetes.

**Methods and analysis:** DPVD is an on-going prospective, multicenter, randomized, double-blind, and placebo-controlled outcome study in individuals with impaired glucose tolerance. Participants, men and women aged  $\geq 30$  years, are randomized to receive eldecalcitol or placebo. They are also given a brief (5 to 10 minutes long) talk about appropriate calorie intake from diet and exercise at each 12-week visit. The primary endpoint is the cumulative incidence of type 2 diabetes. Secondary endpoints include the improving ratio from impaired glucose tolerance to normoglycemia at 48, 96, and 144 weeks. Follow-up is estimated to span 144 weeks.

**Ethics and dissemination:** All protocols and an informed consent form comply with the Ethics Guideline for Clinical Research (Japan Ministry of Health, Labor and Welfare). The study protocol is approved by the Institution Review Board at Kokura Medical Association and University of Occupational and Environmental Health. The study will be implemented in line with the CONSORT statement.

**Trial registration number:** UMIN Clinical Trials Registry: UMIN000010758

### Strengths and limitations of this study:

- This study will elucidate the effect of eldecalcitol, active vitamin D, to prevent the incidence of type 2 diabetes in individuals with impaired glucose tolerance.
- A randomized, double-blinded, placebo-controlled design.
- The drug compliance and the follow-up ratio will be better than those in other trials, where study participants, almost healthy people, receive vitamin D supplements once a year, because our study participants are at high risk for diabetes, all the participants visit clinics to be followed by physicians every 3 months, and they are prescribed active vitamin D formulations at that visit,
- It is unknown whether the results of this study are adapted to other ethnicity because this study consists of only Japanese participants,

INTRODUCTION

Type 2 diabetes is an important risk factor for cardiovascular disease and causes a variety of complications. Moreover, the incidence of type 2 diabetes is currently increasing and has become a major burden to healthcare systems and societies worldwide<sup>1</sup>. In Japan, there are approximately 9.5 million people with diabetes and 109,000 persons per year are diagnosed with diabetes<sup>2</sup>. Additionally, 552 million people worldwide will have type 2 diabetes by the year 2030<sup>3</sup>. The incidence of type 2 diabetes from prediabetes [impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT)] is about 10% (5.8–13.2%) per year<sup>4–7</sup>. While diabetes is an irreversible state, prediabetes can be reverted to a normal glucose state. Therefore, many clinical trials have aimed to reduce the incidence of type 2 diabetes by targeting individuals with prediabetes.

Active vitamin D is currently prescribed as the drug of choice for osteoporosis and hypocalcemia, but it has been reported that vitamin D and active vitamin D might have additional metabolic effects on tissues other than bone and calcium metabolism<sup>8–10</sup> because vitamin D receptors have been found in various tissues, such as brain, pancreas, breast, kidney, colon, prostate, and immune cells<sup>11–14</sup>. One of the additional effects of vitamin D is expected on the glucose metabolism. Vitamin D is regarded to have two mechanisms, by which glucose metabolism in subjects with glucose intolerance will be modulated, *i.e.*, a direct effect on pancreatic  $\beta$  cells and an enhancing effect on insulin sensitivity in insulin target organs. Firstly, vitamin D receptors and 1- $\alpha$ -hydroxylase, which activates the synthesis of active vitamin D [1, 25(OH)<sub>2</sub>D<sub>3</sub>], are expressed in pancreatic  $\beta$  cells<sup>15,16</sup>. Therefore, it is reported that active vitamin D is involved in insulin biosynthesis<sup>17–19</sup>. Secondly, in insulin target organs, such as adipose tissue and skeletal muscle, the expression of the insulin receptor is enhanced (induced?) by active vitamin D in cultured cells<sup>20</sup>. Also, it is reported that vitamin D modulates the activation of PPAR $\delta$ , which is one of the transcription factors controlling lipid metabolism in adipocytes and skeletal muscle<sup>21</sup>.

Many observational studies suggested that the serum 25-hydroxy vitamin D<sub>3</sub> [25(OH)D<sub>3</sub>] level is inversely associated with the incidence of type 2 diabetes<sup>22–26</sup>. Thus, some small-scale clinical trials have

evaluated whether vitamin D and active vitamin D have the effect of improving insulin resistance and glucose metabolism, but the results are still controversial<sup>27-33</sup>.

With the aim of further addressing this issue, we designed a randomized controlled trial to evaluate whether active vitamin D, eldecaltitol, can prevent the incidence of type 2 diabetes among individuals with IGT and whether it can normalize blood glucose levels.

### Objectives and endpoints

The objective of the present study is to elucidate the therapeutic effect of eldecaltitol (active vitamin D) on the prevention of type 2 diabetes among individuals with IGT. The primary endpoint is the hazard ratio of type 2 diabetes onset during 144 weeks of the study period. The secondary endpoints include that the improving ratio from IGT to normoglycemia at 48, 96, and 144 weeks.

## METHODS AND ANALYSES

### Study design

This Diabetes Prevention with active Vitamin D (DPVD) study is designed as a prospective, multicenter, randomized, double-blind, placebo-controlled, and parallel group comparison study. In total, 750 adults with IGT will be randomly assigned to either the active vitamin D or control group, and treated until the onset of type 2 diabetes or the end of treatment period.

### Participant eligibility

Individuals with IGT who meet all inclusion criteria and none of the exclusion criteria (Table 1) are enrolled.

### Intervention

After confirming that an individual conforms to the inclusion and not to the exclusion criteria, a

sub-investigator obtains written informed consent from him/her. The sub-investigator fills out the participants' recognition code list with the date of acquisition of the informed consent, the participant registration number (PRN), and personal information necessary for identifying the PRN with the participant. The PRN is composed of name of the institution, sex (male [M] or female [F]), age (30–54 years [Y]; ≥ 55 years [E]), 75-g OGTT 2h-PG (< 170 mg/dl [L]; ≥170 mg/dl ≤ [H]), and the number of visits. For example, in a hospital, a male participant, aged 43 years, with 176 mg/dl of 2h-PG, who was the fifth participant recruited in this study would have a PRN of hospital name and MYH5.

Randomization list is made by a responsible person in the assignment center at Kokura Medical Association with sex, age, and 75-g OGTT 2h-PG as stratification factors using a permuted block procedure prior to the first patient entry. The list will be kept in a safe in the assignment center until it is time to open the safe. The location of the key will not be disclosed to the investigators or sub-investigators. The key will be retrieved only after the trial is finished and data are fixed, except in the case of emergencies. After confirming eligibility, a staff member in the assignment center allocates a participant to either the active vitamin D group or the control group using central randomization method.

Active vitamin D group: Participants receive 0.75 µg of eldecacitol daily for 144 weeks or until the incidence of diabetes. Eldecacitol is an active vitamin D<sub>3</sub>, which is added with the hydroxypropyloxy group to the position of 2β in calcitriol [1, 25(OH)<sub>2</sub>D<sub>3</sub>], leading to an enhanced calcium absorption from the intestine. Control group: Participants receive one capsule of placebo daily for 144 weeks or until the incidence of diabetes. Participants in both groups are seen at 12-week intervals by their sub-investigators. A brief (5 to 10 minutes long) talk on appropriate calorie intake from diet (ideal body weight × 25–30 kcal) and exercise will be given to each participant based on the common sheet.

**Outline of the study schedule (Table 2)**

After registration and assignment to treatments, all participants are seen at 12-week intervals by their sub-investigators. Body height, body weight and blood pressure are measured, blood samples are taken at baseline and every 12 weeks thereafter. A list of blood tests is shown in Table 3. A 75-g OGTT is

performed at baseline and annually. Serum 25(OH)D levels are measured by using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system<sup>34</sup> at baseline and annually. The system consisted of a Waters Alliance 2795 HPLC interfaced to a Xevo TQ-S (Waters, Milford, MA). Bone mineral density (BMD) is not an obligatory measurement but will be evaluated upon request from participants. If fasting plasma glucose (FPG)  $\geq 126$  mg/dl or HbA<sub>1c</sub>  $\geq 6.5\%$  ( $\geq 48$  mmol/mol) is identified, a 75-g OGTT will be performed within 4 weeks. When HbA<sub>1c</sub>  $\geq 6.5\%$  ( $\geq 48$  mmol/mol) and at least one of the following is recognized, the participant is diagnosed with diabetes: FPG  $\geq 126$  mg/dl, 75-g OGTT 2h-PG  $\geq 200$  mg/dl, or casual plasma glucose (PG)  $\geq 200$  mg/dl<sup>35</sup>. The participants with diabetes will be excluded from the followed-up data analysis. Conversely, when the participants achieve normoglycemia, defined as meeting all three glycemic criteria [FPG  $< 110$  mg/dl, 2h-PG  $< 140$  mg/dl, and HbA<sub>1c</sub>  $< 6.5\%$  ( $< 48$  mmol/mol)] or both FPG  $< 100$  mg/dl and HbA<sub>1c</sub>  $< 5.7\%$  ( $< 42$  mmol/mol)<sup>36</sup> are recorded successively at least twice, the date will be recorded, and they will be followed-up as analysis subjects until diabetes onset. The follow-up period for all participants is 144 weeks.

### Primary endpoint

The primary endpoint is the hazard ratio of type 2 diabetes onset during 144 weeks of the study period: Active vitamin D group vs. Control group. We consider that the comparison of the treatment effect on type 2 diabetes onset between the two groups is the best measurement to evaluate the improvement of insulin resistance and glucose tolerance.

### Secondary endpoints

- 1) Improving ratio from IGT to normoglycemia at 48, 96, and 144 weeks.
- 2) Hazard ratios and 95% confidence intervals (CI) of type 2 diabetes onset in each subgroup at baseline: age ( $\geq$  /  $< 65$  years), sex (men/women), presence or absence of hypertension (systolic  $\geq 140$  mmHg and/or diastolic  $\geq 90$  mmHg), dyslipidemia (LDL-cholesterol  $\geq 140$  g/dl and/or triglycerides  $\geq 150$  mg/dl), obesity (body mass index  $\geq$  /  $< 25$  kg/m<sup>2</sup>), family history of diabetes, FPG ( $\geq$  /  $< 110$  mg/dl), 2h-PG

( $\geq$  /  $<$ 170 mg/dl), 25(OH)D<sub>3</sub> ( $\geq$  /  $<$ 25 ng/ml), homeostasis model assessment of insulin resistance (HOMA-R) ( $\sim$ 1.6 / 1.61–2.49 / 2.5~), and insulinogenic index ( $\geq$  /  $<$ 0.4).

- 3) Hazard ratios and 95% CI of type 2 diabetes development after adjusting for confounding factors: age ( $\geq$  /  $<$ 65 years), sex (men/women), presence or absence of hypertension (systolic  $\geq$ 140 mmHg and/or diastolic  $\geq$ 90 mmHg), dyslipidemia (LDL-cholesterol  $\geq$ 140 g/dl and/or triglycerides  $\geq$ 150 mg/dl), obesity (body mass index  $\geq$  /  $<$ 25 kg/m<sup>2</sup>), family history of diabetes, FPG ( $\geq$  /  $<$ 110 mg/dl), 2h-PG ( $\geq$  /  $<$ 170 mg/dl), 25(OH)D<sub>3</sub> ( $\geq$  /  $<$ 25 ng/ml), HOMA-R ( $\geq$  /  $<$ 2.5), insulinogenic index ( $\geq$  /  $<$ 0.4).
- 4) Hazard ratios of the incidence of adverse events
- 5) Amount and percentage change in HOMA-R during the 144 weeks
- 6) Changes in serum levels of the receptor activator of the nuclear factor kappa B ligand (RANKL), osteoprotegerin, osteocalcin, and leptin during 48 weeks and the association of these items with insulin resistance [HOMA-R and quantitative insulin sensitivity check index (QUICKI)]

**Statistical analyses**

Intention-to-treat population comprises all participants who undergo randomization and receive at least one dose of study drug, and will be analyzed for the assessment of effectiveness and safety. In this analysis plan, the data set is called the full analysis set. Per protocol set comprises all participants who undergo randomization and have no violation of inclusion and exclusion criteria, and this set will be analyzed as the secondary population in the sensitivity analysis. When summing data in each specified period, those data that are obtained beyond an acceptable range of the prescribed observation date or obtained by other than the default method or condition will be treated as missing data. Basic statistics (mean, standard deviation) are calculated for all continuous variables in each treatment arm and each visiting time. If necessary, basic statistics are calculated as a whole. As to FPG, HbA1c, and other laboratory data, differences between the values at baseline and those taken every 12 weeks, and their percent changes from baseline are calculated. Basic statistics, standard errors, or 95% CI are also calculated in these items. The distribution will be summarized in contingency tables for each treatment

arm and each visiting time for all categorical variables. All significance tests and CI were two-sided and performed or constructed at the 5% significance level.

### Sample size

Sample size was calculated with an 80% power ( $1-\beta$ ), a 0.05  $\alpha$  error, and a two-sided test for Log-rank test in Kaplan-Meier survival analysis. Based on our preliminary study (unpublished), we assumed that the cumulative incidence of type 2 diabetes in the control group is 23.14% (8.4% annually) and that in the active vitamin D group is 14.81% (5.2% annually) during 3 years. Thus, the relative risk reduction and drop-out ratio are assumed at 36% and 7%, respectively. As a result, 375 participants are required in each group (total 750).

### Interim analysis

According to the incidence of diabetes, it is assumed that at least 133 participants will develop diabetes during the study period. The total number of interim analyses will be two. When 1 year has passed after the registration of the last participant, the first interim analysis will be conducted. Simultaneously, the drop-out ratio of participants will be confirmed. If it is more than 3% per year, recruitment of trial participants will be conducted again. The second interim analysis will be done when 80 participants develop diabetes, that is, 60% of the expected diabetes incidence. Regarding the primary endpoint, when the difference, determined by the O'Brien-Fleming method, between the both groups is  $< 0.0005$  (in first interim analysis),  $< 0.014$  (in the second interim analysis), or  $< 0.045$  (this is not in an interim analysis but the final analysis)<sup>37</sup>, early completion of the trial will be decided by the Independent Monitoring Committee.

### Ethical considerations and dissemination

This study is being conducted according to Good Clinical Practice (GCP) and the Declaration of Helsinki<sup>38</sup>. It is carried out under the surveillance and guidance of the independent unit at University of

Occupational and Environmental Health in compliance with the ICH-GCP guidelines. The study protocol was approved by the Institutional Review Board at Kokura Medical Association and University of Occupational and Environmental Health. A written informed consent was obtained from each participant before study participation. Participants were informed of their right to withdraw from the study at any time. This study was registered at the University Hospital Medical Information Network clinical trials registry (UMIN000010758). The study results will be presented at national and international conferences, and submitted for publication in peer-reviewed journals.

**DISCUSSION**

There is increasing interest in the potential health benefits by taking vitamin D supplements or active vitamin D formulations. Recent research suggests that vitamin D deficiency may cause not only bone diseases but also a range of non-skeletal diseases, such as cardiovascular diseases, cancers (eg. colorectal, breast), type 2 diabetes, infectious diseases (eg. influenza, common cold, tuberculosis), and autoimmune diseases (eg. multiple sclerosis, type 1 diabetes)<sup>9,39</sup>. However, most of these data come from observational studies, and clinical trial data on the effects of vitamin D are scarce and inconsistent<sup>31-33</sup>. Hence, we focused on the effectiveness of eldecalcitol, active vitamin D, on the incidence of type 2 diabetes among individuals at high risk for diabetes and designed the protocol of DPVD study. This study will provide valuable information, both on the incidence of type 2 diabetes and the improvement to the normal glucose state from IGT.

As a result of necessary compromises of study design, the DPVD study protocol has a number of strengths and weaknesses. Because this study consists of only Japanese participants, it is unknown whether the results of this study are adapted to other ethnicity. Several large-scale clinical trials to examine the benefits of vitamin D in a number of chronic diseases has recently been started around the world: U.S., Finland, New Zealand, 8 European cities, and U.K.<sup>39,40</sup> The primary endpoints are cancer, cardiovascular disease, respiratory disease, infection, hypertension, bone fracture, cognitive

function, and longevity. However, there is no trial of which primary endpoint is diabetes. As compared with these trials in which the number of participants is 2,000 to 20,000, that in our study is 750. However, our study is not considered to be inferior to others because of the following reasons. In other trials, primary endpoints are diverse; study participants are almost healthy person; there is no regular outpatient follow-up by a physician; vitamin D supplements are delivered by a mail once a year. Whereas, in our study, there is one primary endpoint; study participants are at high risk for diabetes; all participants visit clinics to be followed by physicians every 3 months; they are prescribed active vitamin D formulations at that visit. Therefore, the drug compliance and the follow-up ratio in our study will be better than those in other trials; additionally, the sample size of our study, 750 participants, will be enough to evaluate the primary endpoint.

Although vitamin D supplements are chosen to be used in most of other trials, active vitamin D formulation is chosen in our study. Humans get vitamin D from exposure to sunlight, from their diet, and from dietary supplements. Vitamin D from skin and diet is metabolized in the liver to 25-hydroxynitamin D; 25-hydroxynitamin D is metabolized in the kidneys by the enzyme 25-hydroxynitamin D-1 $\alpha$ -hydroxylase to its active form, 1,25-dihydroxynitamin D. Vitamin D itself does not have physiological effect but 1,25-dihydroxynitamin D has : it promotes intestinal calcium absorption in the small intestine and calcium resorption from bone by interacting with the vitamin D receptor to enhance the expression of the epithelial calcium channel and calcium- binding protein<sup>11</sup>. As a result, the administration of active vitamin D rather than vitamin D leads to expression of these physiological effects more effectively and promptly on not only bone and calcium metabolism but also non-skeletal tissue and glucose metabolism. Hence, we chose active vitamin D formulation in the present study. In conclusion, results from this study will provide a clearer picture of the risk and benefits of eldecaciol (active vitamin D) for prevention of type 2 diabetes, and contribute to the resolution of a debate “whether vitamin D and active vitamin D is panacea for glucose metabolism<sup>41</sup>.”

**Current study status**

The DPVD study began recruiting participants in June 2013 and we are still collecting data. It is expected to be reported in 2017.

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**Contributors**

TK, GS, SM, and FK conceived and designed the study. TK drafted the protocol of the study and organized study implementation. TK, GS, TI, SM, FK, YO, and YT refined the study protocol and study implementation. SM and FK conducted the statistical analyses. TK, GS, TI, SM and YT have drafted the manuscript. All authors have read and approved the final version of the manuscript.

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**Conflict of interest**

The authors declare that they have no conflict of interest.

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**Table 1. Eligibility criteria for participation in DPVD study**

Diagnostic criteria of IGT	FPG < 126 mg/dl, 75-g OGTT's 2h-PG is $140 \leq 2h-PG < 200$ mg/dl, and HbA1c < 6.5% (<48 mmol/mol). Additionally, he/she should not be medicated with any antidiabetic drug.
Inclusion criteria	Men and women aged $\geq 30$ years Individuals who are diagnosed with IGT. Serum calcium (corrected value): < 11.0 mg/dl
Exclusion criteria	Individuals who have participated in other clinical trials. Individuals who have been treated with active vitamin D, vitamin D supplement, and/or calcium preparation during last three months. Individuals who have already been diagnosed with type 1 or 2 diabetes. Individuals who have already been initiated with a drug treatment for pre-diabetes. Individuals who are pregnant or have severe diseases, such as renal insufficiency (serum creatinine > 1.5 mg/dl), hepatic insufficiency, psychosis, collagen disease, heart diseases, cerebrovascular diseases. A sub-investigator decides which participants' conditions are inappropriate and preclude his/her participation in the study.

IGT, impaired glucose tolerance; FPG, fasting plasma glucose; 75-g OGTT, 75-g oral glucose tolerance test; 2h-PG, 2-hour plasma glucose.

**Table 2. Outline of the study schedule**

	enrollment	allocation	12, 24, 36 weeks	48 weeks	60, 72, 84 weeks	96 weeks	108, 120, 132 weeks	144 weeks
Time point	-t <sub>1</sub>	t <sub>0</sub>	t <sub>1</sub> , t <sub>2</sub> , t <sub>3</sub>	t <sub>4</sub>	t <sub>5</sub> , t <sub>6</sub> , t <sub>7</sub>	t <sub>8</sub>	t <sub>9</sub> , t <sub>10</sub> , t <sub>12</sub>	t <sub>13</sub>
Enrollment								
Eligibility screen	X							
Informed consent	X							
Allocation		X						
Interventions								
Active vitamin D group								
Eldecalcitol 0.75µg /day								
Control group								
Placebo 1 capsule /day								
Assessments								
Drug compliance			X	X	X	X	X	X
Body height, weight, and blood pressure	X		X	X	X	X	X	X
Blood tests	X		X	X	X	X	X	X
75-g OGTT	X			X		X		X
25(OH)D	X			X		X		X
Bone Mineral Density *		X		X		X		X
Additional laboratory check †		X		X				
Safety monitoring								
Adverse event reporting								

\* Bone mineral density is measured by using dual-energy X-ray absorptiometry at baseline and every 48weeks if a participant wishes.

† Additional laboratory check includes serum levels of receptor activator of nuclear factor kappa B ligand (RANKL), osteoprotegerin, osteocalcin, and leptin.

75-g OGTT, 75-g oral glucose tolerance test; 25(OH)D, 25-hydroxy vitamin D.

**Table 3. Blood tests**

Sampling period	Analysis
Baseline and every 12-week	Fasting plasma glucose or Casual plasma glucose, HbA1c, IRI (glucose tolerance)
	White blood cell, Red blood cell, Hematocrit, Hemoglobin, Platelet (blood counting)
	Sodium, Potassium, Chlorine, Calcium, Phosphorus, Magnesium (serum electrolytes)
	LDL-cholesterol, HDL-cholesterol, Triglycerides (lipids)
	AST, ALT, $\gamma$ -GTP, Proteins, Albumin (liver function)
Baseline and every 48-week	Blood urea nitrogen, Creatinine, Uric acid (renal function)
	75-g OGTT includes IRI (glucose tolerance)
	25(OH)D (vitamin D level)
Baseline and 48-week	RANKL, Osteoprotegerin, Osteocalcin, Leptin (additional laboratory check)

HbA1c, glycated hemoglobin; IRI, immunoreactive insulin; LDL, low density lipoprotein; HDL, high density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase;  $\gamma$ -GTP,  $\gamma$ -Glutamyltranspeptidase; 75-g OGTT, 75-g oral glucose tolerance; 25(OH)D, 25-hydroxy vitamin D; RANKL, receptor activator of nuclear factor kappa B ligand.



# 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	___1___
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	___2 and 9___
	2b	All items from the World Health Organization Trial Registration Data Set	___2___
Protocol version	3	Date and version identifier	___N/A___
Funding	4	Sources and types of financial, material, and other support	___11___
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	___1 and 11___
	5b	Name and contact information for the trial sponsor	___1___
	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	___11___
	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)	___5 and 8___

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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	3
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	4
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)	4

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	N/A
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)	4 and Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	4, 5, and Table 2
	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)	N/A
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	N/A
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
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	6
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)	5 and Table 2

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 8

Recruitment 15 Strategies for achieving adequate participant enrolment to reach target sample size N/A

### Methods: Assignment of interventions (for controlled trials)

#### Allocation:

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 5

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 5

Implementation 16c Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions 5

Blinding (masking) 17a Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how 5

17b If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial 5

### Methods: Data collection, management, and analysis

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 5

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 5

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3	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	5
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7	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	7
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10		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	7
11				
12		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)	7
13				
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16	Methods: Monitoring			
17				
18	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	8
19				
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23		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	8
24				
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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	N/A
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29	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	8
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33	Ethics and dissemination			
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35	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	8
36				
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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)	N/A
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Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	4
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
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	5
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	10
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	11
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	N/A
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	9
	31b	Authorship eligibility guidelines and any intended use of professional writers	11
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
<b>Appendices</b>			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
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	Table 3

\*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

## Rationale and design of Diabetes Prevention with active Vitamin D (DPVD): a randomized, double-blind, placebo-controlled study

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**Rationale and design of Diabetes Prevention with active Vitamin D  
(DPVD): a randomized, double-blind, placebo-controlled study**

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## ABSTRACT

**Introduction:** Recent research suggests that vitamin D deficiency may cause not only bone diseases but also a range of non-skeletal diseases. However, most of these data come from observational studies, and clinical trial data of the effects of vitamin D supplementation on individuals with prediabetes are scarce and inconsistent. The aim of Diabetes Prevention with active Vitamin D (DPVD) study is to assess the effect of eldecacitol, active vitamin D analog, on the incidence of type 2 diabetes among individuals with prediabetes.

**Methods and analysis:** DPVD is an on-going prospective, multicenter, randomized, double-blind, and placebo-controlled outcome study in individuals with impaired glucose tolerance. Participants, men and women aged  $\geq 30$  years, are randomized to receive eldecacitol or placebo. They are also given a brief (5 to 10 minutes long) talk about appropriate calorie intake from diet and exercise at each 12-week visit. The primary endpoint is the cumulative incidence of type 2 diabetes. Secondary endpoints include the improving ratio from impaired glucose tolerance to normoglycemia at 48, 96, and 144 weeks. Follow-up is estimated to span 144 weeks.

**Ethics and dissemination:** All protocols and an informed consent form comply with the Ethics Guideline for Clinical Research (Japan Ministry of Health, Labor and Welfare). The study protocol is approved by the Institution Review Board at Kokura Medical Association and University of Occupational and Environmental Health. The study will be implemented in line with the CONSORT statement.

**Trial registration number:** UMIN Clinical Trials Registry: UMIN000010758

### **Strengths and limitations of this study:**

- This study will elucidate the effect of eldecacitol, active vitamin D, to prevent the incidence of type 2 diabetes in individuals with impaired glucose tolerance.
- A randomized, double-blinded, placebo-controlled design.
- The drug compliance and the follow-up ratio will be better than those in other trials because our study participants are at high risk for diabetes, all the participants visit clinics to be followed by physicians every 12 weeks.

INTRODUCTION

Type 2 diabetes is an important risk factor for cardiovascular disease and causes a variety of complications. Moreover, the incidence of type 2 diabetes is currently increasing and has become a major burden to healthcare systems and societies worldwide<sup>1</sup>. In Japan, there are approximately 9.5 million people with diabetes and 109,000 persons per year are diagnosed with diabetes<sup>2</sup>. Additionally, 552 million people worldwide will have type 2 diabetes by the year 2030<sup>3</sup>. The incidence of type 2 diabetes from prediabetes [impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT)] is about 10% (5.8–13.2%) per year<sup>4-7</sup>. While diabetes is an irreversible state, prediabetes can be reverted to a normal glucose state. Therefore, many clinical trials have aimed to reduce the incidence of type 2 diabetes by targeting individuals with prediabetes.

Active vitamin D is currently prescribed as the drug of choice for osteoporosis and hypocalcemia, but it has been reported that vitamin D and active vitamin D might have additional metabolic effects on tissues other than bone and calcium metabolism<sup>8-10</sup> because vitamin D receptors have been found in various tissues, such as brain, pancreas, breast, kidney, colon, prostate, and immune cells<sup>11-14</sup>. One of the additional effects of vitamin D is expected on the glucose metabolism. Vitamin D is regarded to have two mechanisms, by which glucose metabolism in subjects with glucose intolerance will be modulated, *i.e.*, a direct effect on pancreatic  $\beta$  cells and an enhancing effect on insulin sensitivity in insulin target organs. Firstly, vitamin D receptors and 1- $\alpha$ -hydroxylase, which activates the synthesis of active vitamin D [1, 25(OH)<sub>2</sub>D<sub>3</sub>], are expressed in pancreatic  $\beta$  cells<sup>15,16</sup>. Therefore, it is reported that active vitamin D is involved in insulin biosynthesis<sup>17-19</sup>. Secondly, in insulin target organs, such as adipose tissue and skeletal muscle, the expression of the insulin receptor is enhanced (induced?) by active vitamin D in cultured cells<sup>20</sup>. Also, it is reported that vitamin D modulates the activation of PPAR $\delta$ , which is one of the transcription factors controlling lipid metabolism in adipocytes and skeletal muscle<sup>21</sup>.

Many observational studies suggested that the serum 25-hydroxy vitamin D<sub>3</sub> [25(OH)D<sub>3</sub>] level is inversely associated with the incidence of type 2 diabetes<sup>22-26</sup>. Thus, some small-scale clinical trials have

evaluated whether vitamin D and active vitamin D have the effect of improving insulin resistance and glucose metabolism, but the results are still controversial<sup>27-33</sup>.

With the aim of further addressing this issue, we designed a randomized controlled trial to evaluate whether active vitamin D, eldecaltitol, can prevent the incidence of type 2 diabetes among individuals with IGT and whether it can normalize blood glucose levels.

### Objectives and endpoints

The objective of the present study is to elucidate the therapeutic effect of eldecaltitol (active vitamin D) on the prevention of type 2 diabetes among individuals with IGT. The primary endpoint is the hazard ratio of type 2 diabetes onset during 144 weeks of the study period. The secondary endpoints include that the improving ratio from IGT to normoglycemia at 48, 96, and 144 weeks.

## METHODS AND ANALYSES

### Study design

This Diabetes Prevention with active Vitamin D (DPVD) study is designed as a prospective, multicenter, randomized, double-blind, placebo-controlled, and parallel group comparison study. In total, 750 adults with IGT will be randomly assigned to either the active vitamin D or control group, and treated until the onset of type 2 diabetes or the end of treatment period. This study is investigator-initiated and the study sponsor is University of Occupational and Environmental Health.

### Study population and settings

Participants will be recruited from outpatient clinics in Fukuoka prefecture and Kanagawa prefecture in Japan by the investigators. Individuals with IGT who meet all inclusion criteria and none of the exclusion criteria (Table 1) are enrolled for randomization. All participants will be followed at University of Occupational and Environmental Health, Kokura Medical Association Health Testing Center, or Fujisawa City Hospital.

**Registration procedure**

After confirming that an individual conforms to the inclusion and not to the exclusion criteria, a sub-investigator obtains written informed consent from him/her. The sub-investigator fills out the participants' recognition code list with the date of acquisition of the informed consent, the participant registration number (PRN), and personal information necessary for identifying the PRN with the participant. The PRN is composed of name of the institution, sex (male [M] or female [F]), age (30–54 years [Y]; ≥ 55 years [E]), 75-g OGTT 2h-PG (< 170 mg/dl [L]; ≥170 mg/dl ≤ [H]), and the number of visits. For example, in a hospital, a male participant, aged 43 years, with 176 mg/dl of 2h-PG, who was the fifth participant recruited in this study would have a PRN of hospital name and MYH5. The sub-investigator also fills out a case report form (CRF) with predetermined requirements and applies for registration and treatment assignment by sending the CRF with the PRN via fax to the Assignment Center at Kokura Medical Association.

**Randomization and blinding**

Participants will be assigned in a 1:1 ratio to either the active vitamin D group or the control group by using a central randomization method. A randomization list is made using a permuted block procedure by a responsible person in the Assignment Center prior to the first participant's entry, and the list is stratified by sex, age, 75-g OGTT 2h-PG, because these factors are considered to affect the incidence of diabetes. The assignment list will be kept in a safe in the Assignment Center until it is time to open the safe. The location of the key will not be disclosed to the investigators or sub-investigators. Therefore, both the study personnel and the participants will be masked to which treatment group. The key will be retrieved only after the trial is finished and data are fixed, except in the case of interim analyses or emergencies.

**Intervention**

Active vitamin D group: Participants receive 0.75 µg of eldecalcitol daily for 144 weeks or until the incidence of diabetes. Eldecalcitol is an active vitamin D<sub>3</sub>, which is added with the hydroxypropyloxy

group to the position of 2 $\beta$  in calcitriol [1, 25(OH) $_2$ D $_3$ ], leading to an enhanced calcium absorption from the intestine. Control group: Participants receive one capsule of placebo daily for 144 weeks or until the incidence of diabetes. Participants in both groups are seen at 12-week intervals by their sub-investigators. A brief (5 to 10 minutes long) talk on appropriate calorie intake from diet (ideal body weight  $\times$  25–30 kcal) and exercise will be given to each participant based on the common sheet.

### Additional treatment criteria

When a participant meets the criteria for additional treatment described below, an additional treatment will be initiated. Sub-investigators will describe the date and reason for the additional treatment in the CRF.

1. If LDL cholesterol levels exceed 160 mg/dl, participants in both groups will be treated with statins.

The sub-investigator can prescribe their statin of choice.

2. If triglyceride levels exceed 220 mg/dl, participants in the both groups will be treated with fibrates.

The sub-investigator can prescribe their fibrate of choice.

3. If BMD decreases to  $\leq -2.5$  SD, participants in both groups will be treated with bisphosphonates.

The sub-investigator can prescribe their bisphosphonate of choice. However, if there is a significant difference between the two groups in the number of participants with bisphosphonates administration, the analysis is to be performed after adjustment.

4. If systolic blood pressure exceeds 150 mmHg and/or diastolic pressure exceeds 100 mmHg, participants in the both groups will be dosed with antihypertensive drugs. The sub-investigator can prescribe their hypertensive drug of choice.

### Discontinuation of treatment

When a participant meets the discontinuation criteria described below, sub-investigators shall stop the intervention. They shall describe the date and reason for the discontinuation on the CRF, and perform the same examinations initially planned to be done after the 144-week treatment to evaluate the efficacy and

safety. If the treatment discontinuation was caused by adverse events, sub-investigators must follow the participant until he/she recovers to the original state as much as possible.

1. A participant withdraws consent for participation
2. Continuation of the study treatment has become difficult because of adverse events
3. A participant is diagnosed with type 2 diabetes
4. It is recognized that a participant did not satisfy eligibility criteria after registration, except for improving to normoglycemia after treatment
5. Serum calcium levels reach  $\geq 11.0$  mg/dl
6. Sub-investigators judge that it is suitable to discontinue the study

**Outline of the study schedule (Table 2)**

After registration and assignment to treatments, all participants are seen at 12-week intervals by their sub-investigators. Body height, body weight and blood pressure are measured, blood samples are taken at baseline and every 12 weeks thereafter. A list of blood tests is shown in Table 3. A 75-g OGTT is performed at baseline and annually. Serum 25(OH)D levels are measured by using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system<sup>34</sup> at baseline and annually. The system consisted of a Waters Alliance 2795 HPLC interfaced to a Xevo TQ-S (Waters, Milford, MA). Bone mineral density (BMD) is not an obligatory measurement but will be evaluated upon request from participants. If fasting plasma glucose (FPG)  $\geq 126$  mg/dl or HbA<sub>1c</sub>  $\geq 6.5\%$  ( $\geq 48$  mmol/mol) is identified, a 75-g OGTT will be performed within 4 weeks. When HbA<sub>1c</sub>  $\geq 6.5\%$  ( $\geq 48$  mmol/mol) and at least one of the following is recognized, the participant is diagnosed with diabetes: FPG  $\geq 126$  mg/dl, 75-g OGTT 2h-PG  $\geq 200$  mg/dl, or casual plasma glucose (PG)  $\geq 200$  mg/dl<sup>35</sup>. The participants with diabetes will be excluded from the followed-up data analysis. Conversely, when the participants achieve normoglycemia, defined as meeting all three glycemic criteria [FPG  $< 110$  mg/dl, 2h-PG  $< 140$  mg/dl, and HbA<sub>1c</sub>  $< 6.5\%$  ( $< 48$  mmol/mol)] or both FPG  $< 100$  mg/dl and HbA<sub>1c</sub>  $< 5.7\%$  ( $< 42$  mmol/mol)<sup>36</sup> are recorded successively at least twice, the date will be recorded, and they will be followed-up as analysis subjects until diabetes

onset. The follow-up period for all participants is 144 weeks.

### Primary endpoint

The primary endpoint is the hazard ratio of type 2 diabetes onset during 144 weeks of the study period: Active vitamin D group vs. Control group. We consider that the comparison of the treatment effect on type 2 diabetes onset between the two groups is the best measurement to evaluate the improvement of insulin resistance and glucose tolerance.

### Secondary endpoints

- 1) Improving ratio from IGT to normoglycemia at 48, 96, and 144 weeks.
- 2) Hazard ratios and 95% confidence intervals (CI) of type 2 diabetes onset in each subgroup at baseline: age ( $\geq$  /  $<$ 65 years), sex (men/women), presence or absence of hypertension (systolic  $\geq$  140 mmHg and/or diastolic  $\geq$  90 mmHg), dyslipidemia (LDL-cholesterol  $\geq$  140 g/dl and/or triglycerides  $\geq$  150 mg/dl), obesity (body mass index  $\geq$  /  $<$ 25 kg/m<sup>2</sup>), family history of diabetes, FPG ( $\geq$  /  $<$ 110 mg/dl), 2h-PG ( $\geq$  /  $<$ 170 mg/dl), 25(OH)D<sub>3</sub> ( $\geq$  /  $<$ 25 ng/ml), homeostasis model assessment of insulin resistance (HOMA-R) ( $\sim$ 1.6 / 1.61–2.49 / 2.5~), and insulinogenic index ( $\geq$  /  $<$ 0.4).
- 3) Hazard ratios and 95% CI of type 2 diabetes development after adjusting for confounding factors: age ( $\geq$  /  $<$ 65 years), sex (men/women), presence or absence of hypertension (systolic  $\geq$  140 mmHg and/or diastolic  $\geq$  90 mmHg), dyslipidemia (LDL-cholesterol  $\geq$  140 g/dl and/or triglycerides  $\geq$  150 mg/dl), obesity (body mass index  $\geq$  /  $<$ 25 kg/m<sup>2</sup>), family history of diabetes, FPG ( $\geq$  /  $<$ 110 mg/dl), 2h-PG ( $\geq$  /  $<$ 170 mg/dl), 25(OH)D<sub>3</sub> ( $\geq$  /  $<$ 25 ng/ml), HOMA-R ( $\geq$  /  $<$ 2.5), insulinogenic index ( $\geq$  /  $<$ 0.4).
- 4) Hazard ratios of the incidence of adverse events
- 5) Amount and percentage change in HOMA-R during the 144 weeks
- 6) Changes in serum levels of the receptor activator of the nuclear factor kappa B ligand (RANKL), osteoprotegerin, osteocalcin, and leptin during 48 weeks and the association of these items with insulin resistance [HOMA-R and quantitative insulin sensitivity check index (QUICKI)]

**Statistical analyses**

Intention-to-treat population comprises all participants who undergo randomization and receive at least one dose of study drug, and will be analyzed for the assessment of effectiveness and safety. In this analysis plan, the data set is called the full analysis set. Per protocol set comprises all participants who undergo randomization and have no violation of inclusion and exclusion criteria, and this set will be analyzed as the secondary population in the sensitivity analysis. When summing data in each specified period, those data that are obtained beyond an acceptable range of the prescribed observation date or obtained by other than the default method or condition will be treated as missing data. Basic statistics (mean, standard deviation) are calculated for all continuous variables in each treatment arm and each visiting time. If necessary, basic statistics are calculated as a whole. As to FPG, HbA1c, and other laboratory data, differences between the values at baseline and those taken every 12 weeks, and their percent changes from baseline are calculated. Basic statistics, standard errors, or 95% CI are also calculated in these items. The distribution will be summarized in contingency tables for each treatment arm and each visiting time for all categorical variables. All significance tests and CI were two-sided and performed or constructed at the 5% significance level.

**Sample size**

Sample size was calculated with an 80% power ( $1-\beta$ ), a 0.05  $\alpha$  error, and a two-sided test for Log-rank test in Kaplan-Meier survival analysis. Based on our preliminary study (unpublished), we assumed that the cumulative incidence of type 2 diabetes in the control group is 23.14% (8.4% annually) and that in the active vitamin D group is 14.81% (5.2% annually) during 3 years. Thus, the relative risk reduction and drop-out ratio are assumed at 36% and 7%, respectively. As a result, 375 participants are required in each group (total 750).

**Interim analyses**

According to the incidence of diabetes, it is assumed that at least 133 participants will develop diabetes

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3 during the study period. The total number of interim analyses will be two. When 1 year has passed after  
4 the registration of the last participant, the first interim analysis will be conducted. Simultaneously, the  
5 drop-out ratio of participants will be confirmed. If it is more than 3% per year, recruitment of trial  
6 participants will be conducted again. The second interim analysis will be done when 80 participants  
7 develop diabetes, that is, 60% of the expected diabetes incidence. Regarding the primary endpoint, when  
8 the difference, determined by the O'Brien-Fleming method, between the both groups is  $< 0.0005$  (in first  
9 interim analysis),  $< 0.014$  (in the second interim analysis), or  $< 0.045$  (this is not in an interim analysis but  
10 the final analysis)<sup>37</sup>, early completion of the trial will be decided by the independent Data Monitoring  
11 Committee.  
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### 25 **Trial management and independent committees**

26 The Trial Steering Committee (Chair: GS) will meet on a termly basis to review status of the overall program,  
27 including trial progress. In the case that they amend the protocol for a legitimate reason, they shall submit a  
28 report about the amendment to the principal investigator and IRB as soon as possible for their review and  
29 approval. The decision of the amendment must be informed to all authorized study personnel immediately.  
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35 The Data Collection Center will collect the accumulating outcome data from all trial participating  
36 institutions. The staff member of the center will clean up and aggregate the data but won't know which group a  
37 participant belongs to.  
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41 The independent Data Monitoring Committee (Chair: TI) will audit the conduct of the trial with safety  
42 and ethics review regularly and conduct the interim analyses. When the trial is finished or the independent  
43 Data Monitoring Committee adjudicate the trial completion according to the result of the interim analyses,  
44 the final dataset will be directly sent to a fully independent Data Analyses Committee (Chair: SM). The  
45 data will be analyzed for all endpoints. Any investigators other than the Data Analyses Committee cannot  
46 access the data.  
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### 57 **Ethical considerations and dissemination**

This study is being conducted according to Good Clinical Practice (GCP) and the Declaration of Helsinki<sup>38</sup>. It is carried out under the surveillance and guidance of the independent Data Monitoring Committee in compliance with the ICH-GCP guidelines. The study protocol was approved by the Institutional Review Board at Kokura Medical Association, University of Occupational and Environmental Health, and Fujisawa City Hospital. A written informed consent was obtained from each participant before study participation. Participants were informed of their right to withdraw from the study at any time. This study was registered at the University Hospital Medical Information Network clinical trials registry (UMIN000010758). The study results will be presented at national and international conferences, and submitted for publication in peer-reviewed journals.

DISCUSSION

There is increasing interest in the potential health benefits by taking vitamin D supplements or active vitamin D formulations. Recent research suggests that vitamin D deficiency may cause not only bone diseases but also a range of non-skeletal diseases, such as cardiovascular diseases, cancers (eg. colorectal, breast), type 2 diabetes, infectious diseases (eg. influenza, common cold, tuberculosis), and autoimmune diseases (eg. multiple sclerosis, type 1 diabetes)<sup>9,39</sup>. However, most of these data come from observational studies, and clinical trial data on the effects of vitamin D are scarce and inconsistent<sup>31-33</sup>. Hence, we focused on the effectiveness of eldecalcitol, active vitamin D, on the incidence of type 2 diabetes among individuals at high risk for diabetes and designed the protocol of DPVD study. This study will provide valuable information, both on the incidence of type 2 diabetes and the improvement to the normal glucose state from IGT.

As a result of necessary compromises of study design, the DPVD study protocol has a number of strengths and weaknesses. Because this study consists of only Japanese participants, it is unknown whether the results of this study are adapted to other ethnicity. Several large-scale clinical trials to examine the benefits of vitamin D in a number of chronic diseases has recently been started around the

world: U.S., Finland, New Zealand, 8 European cities, and U.K.<sup>39,40</sup> The primary endpoints are cancer, cardiovascular disease, respiratory disease, infection, hypertension, bone fracture, cognitive function, and longevity. However, there is no trial of which primary endpoint is diabetes. As compared with these trials in which the number of participants is 2,000 to 20,000, that in our study is 750. However, our study is not considered to be inferior to others because of the following reasons. In other trials, primary endpoints are diverse; study participants are almost healthy person; there is no regular outpatient follow-up by a physician; vitamin D supplements are delivered by a mail once a year. Whereas, in our study, there is one primary endpoint; study participants are at high risk for diabetes; all participants visit clinics to be followed by physicians every 3 months; they are prescribed active vitamin D formulations at that visit. Therefore, the drug compliance and the follow-up ratio in our study will be better than those in other trials; additionally, the sample size of our study, 750 participants, will be enough to evaluate the primary endpoint.

Although vitamin D supplements are chosen to be used in most of other trials, active vitamin D formulation is chosen in our study. Humans get vitamin D from exposure to sunlight, from their diet, and from dietary supplements. Vitamin D from skin and diet is metabolized in the liver to 25-hydroxynitamin D; 25-hydroxynitamin D is metabolized in the kidneys by the enzyme 25-hydroxynitamin D-1 $\alpha$ -hydroxylase to its active form, 1,25-dihydroxynitamin D. Vitamin D itself does not have physiological effect but 1,25-dihydroxynitamin D has : it promotes intestinal calcium absorption in the small intestine and calcium resorption from bone by interacting with the vitamin D receptor to enhance the expression of the epithelial calcium channel and calcium- binding protein<sup>11</sup>. As a result, the administration of active vitamin D rather than vitamin D leads to expression of these physiological effects more effectively and promptly on not only bone and calcium metabolism but also non-skeletal tissue and glucose metabolism. Hence, we chose active vitamin D formulation in the present study. In conclusion, results from this study will provide a clearer picture of the risk and benefits of eldecaciol (active vitamin D) for prevention of type 2 diabetes, and contribute to the resolution of a debate “whether vitamin D and active vitamin D is panacea for glucose metabolism<sup>41</sup>.”

**Current study status**

The DPVD study began recruiting participants in June 2013 and we are still collecting data. It is expected to be reported in 2017.

**Acknowledgements**

We are grateful to all study participants and all staff of this study for recruiting the participants and contributing to the accurate recording of trial data.

**Contributors**

TK, GS, SM, and FK conceived and designed the study. TK drafted the protocol of the study and organized study implementation. TK, GS, TI, SM, FK, YO, and YT refined the study protocol and study implementation. SM and FK conducted the statistical analyses. TK, GS, TI, SM and YT have drafted the manuscript. All authors have read and approved the final version of the manuscript.

**Funding**

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**Conflict of interest**

The authors declare that they have no conflict of interest.

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**Table 1. Eligibility criteria for participation in DPVD study**

Diagnostic criteria of IGT	FPG < 126 mg/dl, 75-g OGTT's 2h-PG is $140 \leq 2h-PG < 200$ mg/dl, and HbA1c < 6.5% (<48 mmol/mol). Additionally, he/she should not be medicated with any antidiabetic drug.
Inclusion criteria	Men and women aged $\geq 30$ years Individuals who are diagnosed with IGT. Serum calcium (corrected value): < 11.0 mg/dl
Exclusion criteria	Individuals who have participated in other clinical trials. Individuals who have been treated with active vitamin D, vitamin D supplement, and/or calcium preparation during last three months. Individuals who have already been diagnosed with type 1 or 2 diabetes. Individuals who have already been initiated with a drug treatment for pre-diabetes. Individuals who are pregnant or have severe diseases, such as renal insufficiency (serum creatinine > 1.5 mg/dl), hepatic insufficiency, psychosis, collagen disease, heart diseases, cerebrovascular diseases. A sub-investigator decides which participants' conditions are inappropriate and preclude his/her participation in the study.

IGT, impaired glucose tolerance; FPG, fasting plasma glucose; 75-g OGTT, 75-g oral glucose tolerance test; 2h-PG, 2-hour plasma glucose.

**Table 2. Outline of the study schedule**

	enrollment	allocation	12, 24, 36 weeks	48 weeks	60, 72, 84 weeks	96 weeks	108, 120, 132 weeks	144 weeks
Time point	-t <sub>1</sub>	t <sub>0</sub>	t <sub>1</sub> , t <sub>2</sub> , t <sub>3</sub>	t <sub>4</sub>	t <sub>5</sub> , t <sub>6</sub> , t <sub>7</sub>	t <sub>8</sub>	t <sub>9</sub> , t <sub>10</sub> , t <sub>12</sub>	t <sub>13</sub>
Enrollment								
Eligibility screen	X							
Informed consent	X							
Allocation		X						
Interventions								
Active vitamin D group								
Eldecalcitol 0.75µg /day								
Control group								
Placebo 1 capsule /day								
Assessments								
Drug compliance			X	X	X	X	X	X
Body height, weight, and blood pressure	X		X	X	X	X	X	X
Blood tests	X		X	X	X	X	X	X
75-g OGTT	X			X		X		X
25(OH)D	X			X		X		X
Bone Mineral Density *		X		X		X		X
Additional laboratory check †		X		X				
Safety monitoring								
Adverse event reporting								

\* Bone mineral density is measured by using dual-energy X-ray absorptiometry at baseline and every 48weeks if a participant wishes.

† Additional laboratory check includes serum levels of receptor activator of nuclear factor kappa B ligand (RANKL), osteoprotegerin, osteocalcin, and leptin.

75-g OGTT, 75-g oral glucose tolerance test; 25(OH)D, 25-hydroxy vitamin D.

**Table 3. Blood tests**

Sampling period	Analysis
Baseline and every 12-week	Fasting plasma glucose or Casual plasma glucose, HbA1c, IRI (glucose tolerance)
	White blood cell, Red blood cell, Hematocrit, Hemoglobin, Platelet (blood counting)
	Sodium, Potassium, Chlorine, Calcium, Phosphorus, Magnesium (serum electrolytes)
	LDL-cholesterol, HDL-cholesterol, Triglycerides (lipids)
	AST, ALT, $\gamma$ -GTP, Proteins, Albumin (liver function)
Baseline and every 48-week	Blood urea nitrogen, Creatinine, Uric acid (renal function)
	75-g OGTT includes IRI (glucose tolerance)
	25(OH)D (vitamin D level)
Baseline and 48-week	RANKL, Osteoprotegerin, Osteocalcin, Leptin (additional laboratory check)

HbA1c, glycated hemoglobin; IRI, immunoreactive insulin; LDL, low density lipoprotein; HDL, high density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase;  $\gamma$ -GTP,  $\gamma$ -Glutamyltranspeptidase; 75-g OGTT, 75-g oral glucose tolerance; 25(OH)D, 25-hydroxy vitamin D; RANKL, receptor activator of nuclear factor kappa B ligand.



# 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	___1___
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	___2 and 11___
	2b	All items from the World Health Organization Trial Registration Data Set	___2___
Protocol version	3	Date and version identifier	___N/A___
Funding	4	Sources and types of financial, material, and other support	___13___
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	___1 and 11___
	5b	Name and contact information for the trial sponsor	___4___
	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	___13___
	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)	___11___

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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	3
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	4
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)	4

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	4
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)	4 and Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	5, 7, and Table 2
	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)	6
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	2 and 6
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
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	8
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)	7 and Table 2

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	____9____
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	____N/A____

### Methods: Assignment of interventions (for controlled trials)

#### Allocation:

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	____5____
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	____5____
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	____5____
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	____5____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	____5____

### Methods: Data collection, management, and analysis

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	____7 and 10____
	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	____9____

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3	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	___1 and 10___
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7	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	___9___
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10		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	___9___
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12		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)	___9___
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16	<b>Methods: Monitoring</b>			
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18	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	___9 and 10___
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23		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	___9___
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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	___8___
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29	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	___10___
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33	<b>Ethics and dissemination</b>			
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35	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	___11___
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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)	___10___
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Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	_____5_____
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	_____N/A_____
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	_____10_____
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_____13_____
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	_____10_____
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	_____N/A_____
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	_____11_____
	31b	Authorship eligibility guidelines and any intended use of professional writers	_____13_____
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____N/A_____
<b>Appendices</b>			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	_____N/A_____
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	_____Table 3_____

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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
Administrative information			
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>1 and 5</u>
Protocol version	3	Date and version identifier	<u>1</u>
Funding	4	Sources and types of financial, material, and other support	<u>28</u>
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	<u>35</u>
	5b	Name and contact information for the trial sponsor	<u>6</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>28</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>10, 15, and 28</u>

**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>7</u>
	6b	Explanation for choice of comparators	<u>8</u>
Objectives	7	Specific objectives or hypotheses	<u>8</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>8</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>8</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>8</u>
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	<u>10</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>14 and 29</u>
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	<u>N/A</u>
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	<u>13</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>15 and 21</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>18 and 20</u>

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>21 and 29</u>
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	<u>N/A</u>
<b>Methods: Assignment of interventions (for controlled trials)</b>			
Allocation:			
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>11</u>
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>10</u>
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	<u>11</u>
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	<u>11</u>
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	<u>11</u>
<b>Methods: Data collection, management, and analysis</b>			
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>13</u>
	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>25</u>

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	<u>10</u>
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	<u>26</u>
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	<u>26</u>
	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)	<u>24</u>
<b>Methods: Monitoring</b>			
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	<u>15</u>
	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	<u>27</u>
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	<u>15</u>
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	<u>15</u>
<b>Ethics and dissemination</b>			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	<u>1 and 9</u>
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)	<u>29</u>

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
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	10
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	28
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	N/A
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	15
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	30
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	N/A (We have a Japanese version.)
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	18

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# BMJ Open

## Rationale and design of Diabetes Prevention with active Vitamin D (DPVD): a randomized, double-blind, placebo-controlled study

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**Rationale and design of Diabetes Prevention with active Vitamin D  
(DPVD): a randomized, double-blind, placebo-controlled study**

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## ABSTRACT

**Introduction:** Recent research suggests that vitamin D deficiency may cause not only bone diseases but also a range of non-skeletal diseases. However, most of these data come from observational studies, and clinical trial data of the effects of vitamin D supplementation on individuals with prediabetes are scarce and inconsistent. The aim of Diabetes Prevention with active Vitamin D (DPVD) study is to assess the effect of eldecacitol, active vitamin D analog, on the incidence of type 2 diabetes among individuals with prediabetes.

**Methods and analysis:** DPVD is an on-going prospective, multicenter, randomized, double-blind, and placebo-controlled outcome study in individuals with impaired glucose tolerance. Participants, men and women aged  $\geq 30$  years, will be randomized to receive eldecacitol or placebo. They will be also given a brief (5 to 10 minutes long) talk about appropriate calorie intake from diet and exercise at each 12-week visit. The primary endpoint is the cumulative incidence of type 2 diabetes. Secondary endpoints include the improving ratio from impaired glucose tolerance to normoglycemia at 48, 96, and 144 weeks. Follow-up is estimated to span 144 weeks.

**Ethics and dissemination:** All protocols and an informed consent form comply with the Ethics Guideline for Clinical Research (Japan Ministry of Health, Labor and Welfare). The study protocol has been approved by the Institutional Review Board at Kokura Medical Association and University of Occupational and Environmental Health. The study will be implemented in line with the CONSORT statement.

**Trial registration number:** UMIN Clinical Trials Registry: UMIN000010758

### **Strengths and limitations of this study:**

- This study will provide new evidence concerning the effect of eldecacitol, active vitamin D, to prevent the incidence of type 2 diabetes in individuals with impaired glucose tolerance.
- Our study participants will be at high risk for diabetes, all the participants will visit clinics to be followed by physicians every 12 weeks, and they will be prescribed active vitamin D formulations at that visit. Therefore, the drug compliance and the follow-up ratio in our study will be better than those in other trials, where study participants, almost healthy people, receive vitamin D supplements once a year.
- It is unknown whether the results of this study applies to other ethnicity because this study consists of only Japanese participants.

INTRODUCTION

Type 2 diabetes is an important risk factor for cardiovascular disease and causes a variety of complications. Moreover, the incidence of type 2 diabetes is currently increasing and has become a major burden to healthcare systems and societies worldwide<sup>1</sup>. In Japan, there are approximately 9.5 million people with diabetes and 109,000 persons per year are diagnosed with diabetes<sup>2</sup>. Additionally, 552 million people worldwide will have type 2 diabetes by the year 2030<sup>3</sup>. The incidence of type 2 diabetes from prediabetes [impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT)] is about 10% (5.8–13.2%) per year<sup>4-7</sup>. While diabetes is an irreversible state, prediabetes can be reverted to a normal glucose state. Therefore, many clinical trials have aimed to reduce the incidence of type 2 diabetes by targeting individuals with prediabetes.

Active vitamin D is currently prescribed as the drug of choice for osteoporosis and hypocalcemia, but it has been reported that vitamin D and active vitamin D might have additional metabolic effects on tissues other than bone and calcium metabolism<sup>8-10</sup> because vitamin D receptors have been found in various tissues, such as brain, pancreas, breast, kidney, colon, prostate, and immune cells<sup>11-14</sup>. One of the additional effects of vitamin D is expected on the glucose metabolism. Vitamin D is regarded to have two mechanisms, by which glucose metabolism in subjects with glucose intolerance will be modulated, *i.e.*, a direct effect on pancreatic  $\beta$  cells and an enhancing effect on insulin sensitivity in insulin target organs. Firstly, vitamin D receptors and 1- $\alpha$ -hydroxylase, which activates the synthesis of active vitamin D [1, 25(OH)<sub>2</sub>D<sub>3</sub>], are expressed in pancreatic  $\beta$  cells<sup>15,16</sup>. Therefore, it is reported that active vitamin D is involved in insulin biosynthesis<sup>17-19</sup>. Secondly, in insulin target organs, such as adipose tissue and skeletal muscle, the expression of the insulin receptor is enhanced by active vitamin D in cultured cells<sup>20</sup>. Also, it is reported that vitamin D modulates the activation of PPAR $\delta$ , which is one of the transcription factors controlling lipid metabolism in adipocytes and skeletal muscle<sup>21</sup>.

Many observational studies suggested that the serum 25-hydroxy vitamin D<sub>3</sub> [25(OH)D<sub>3</sub>] level was inversely associated with the incidence of type 2 diabetes<sup>22-26</sup>. Thus, some small-scale clinical trials have

evaluated whether vitamin D and active vitamin D have the effect of improving insulin resistance and glucose metabolism, but the results are still controversial<sup>27-33</sup>.

With the aim of further addressing this issue, we designed a randomized controlled trial to evaluate whether active vitamin D, eldecaltitol, can prevent the incidence of type 2 diabetes among individuals with IGT and whether it can normalize blood glucose levels.

### Objectives and endpoints

The objective of the present study is to elucidate the therapeutic effect of eldecaltitol (active vitamin D) on the prevention of type 2 diabetes among individuals with IGT. The primary endpoint is the hazard ratio of type 2 diabetes onset during 144 weeks of the study period. The secondary endpoints include that the improving ratio from IGT to normoglycemia at 48, 96, and 144 weeks.

## METHODS AND ANALYSES

### Study design

This Diabetes Prevention with active Vitamin D (DPVD) study is designed as a prospective, multicenter, randomized, double-blind, placebo-controlled, and parallel group comparison study. In total, 750 adults with IGT will be randomly assigned to either the active vitamin D or control group, and treated until the onset of type 2 diabetes or the end of treatment period. This study is investigator-initiated and the study sponsor is University of Occupational and Environmental Health.

### Study population and settings

Participants will be recruited from outpatient clinics in Fukuoka prefecture and Kanagawa prefecture in Japan by the investigators. Individuals with IGT who meet all inclusion criteria and none of the exclusion criteria (Table 1) will be enrolled for randomization. All participants will be followed at University of Occupational and Environmental Health, Kokura Medical Association Health Testing Center, or Fujisawa City Hospital.

**Registration procedure**

After confirming that an individual conforms to the inclusion and not to the exclusion criteria, a sub-investigator will obtain written informed consent from him/her. The sub-investigator will fill out the participants' recognition code list with the date of acquisition of the informed consent, the participant registration number (PRN), and personal information necessary for identifying the PRN with the participant. The PRN is composed of name of the institution, sex (male [M] or female [F]), age (30–54 years [Y]; ≥ 55 years [E]), 75-g OGTT 2h-PG (< 170 mg/dl [L]; ≥170 mg/dl ≤ [H]), and the number of visits. For example, in a hospital, a male participant, aged 43 years, with 176 mg/dl of 2h-PG, who is the fifth participant recruited in this study will have a PRN of hospital name and MYH5. The sub-investigator will also fill out a case report form (CRF) with predetermined requirements and applies for registration and treatment assignment by sending the CRF with the PRN via fax to the Assignment Center at Kokura Medical Association.

**Randomization and blinding**

Participants will be assigned in a 1:1 ratio to either the active vitamin D group or the control group by using a central randomization method. A randomization list is made using a permuted block procedure by a responsible person in the Assignment Center prior to the first participant's entry, and the list is stratified by sex, age, 75-g OGTT 2h-PG, because these factors are considered to affect the incidence of diabetes. The assignment list will be kept in a safe in the Assignment Center until it is time to open the safe. The location of the key will not be disclosed to the investigators or sub-investigators. Therefore, both the study personnel and the participants will be masked to which treatment group. The key will be retrieved only after the trial is finished and data are fixed, except in the case of interim analyses or emergencies.

**Intervention**

Active vitamin D group: Participants will receive 0.75 µg of eldecacitol daily for 144 weeks or until the incidence of diabetes. Eldecacitol is an active vitamin D<sub>3</sub>, which is added with the hydroxypropyloxy

group to the position of 2 $\beta$  in calcitriol [1, 25(OH) $_2$ D $_3$ ], leading to an enhanced calcium absorption from the intestine. Control group: Participants will receive one capsule of placebo daily for 144 weeks or until the incidence of diabetes. Participants in both groups will be seen at 12-week intervals by their sub-investigators. A brief (5 to 10 minutes long) talk on appropriate calorie intake from diet (ideal body weight  $\times$  25–30 kcal) and exercise will be given to each participant based on the common sheet.

### Additional treatment criteria

When a participant meets the criteria for additional treatment described below, an additional treatment will be initiated. Sub-investigators will describe the date and reason for the additional treatment in the CRF.

1. If LDL cholesterol levels exceed 160 mg/dl, participants in both groups will be treated with statins.

The sub-investigator can prescribe their statin of choice.

2. If triglyceride levels exceed 220 mg/dl, participants in the both groups will be treated with fibrates.

The sub-investigator can prescribe their fibrate of choice.

3. If BMD decreases to  $\leq -2.5$  SD, participants in both groups will be treated with bisphosphonates.

The sub-investigator can prescribe their bisphosphonate of choice. However, if there is a significant difference between the two groups in the number of participants with bisphosphonates administration, the analysis is to be performed after adjustment.

4. If systolic blood pressure exceeds 150 mmHg and/or diastolic pressure exceeds 100 mmHg, participants in the both groups will be dosed with antihypertensive drugs. The sub-investigator can prescribe their hypertensive drug of choice.

### Discontinuation of treatment

When a participant meets the discontinuation criteria described below, sub-investigators shall stop the intervention. They shall describe the date and reason for the discontinuation on the CRF, and perform the same examinations initially planned to be done after the 144-week treatment to evaluate the efficacy and

safety. If the treatment discontinuation is caused by adverse events, sub-investigators will have to follow the participant until he/she recovers to the original state as much as possible.

1. A participant withdraws consent for participation
2. Continuation of the study treatment has become difficult because of adverse events
3. A participant is diagnosed with type 2 diabetes
4. It is recognized that a participant did not satisfy eligibility criteria after registration, except for improving to normoglycemia after treatment
5. Serum calcium levels reach  $\geq 11.0$  mg/dl
6. Sub-investigators judge that it is suitable to discontinue the study

**Outline of the study schedule (Table 2)**

After registration and assignment to treatments, all participants will be seen at 12-week intervals by their sub-investigators. Body height, body weight and blood pressure will be measured, blood samples will be taken at baseline and every 12 weeks thereafter. A list of blood tests is shown in Table 3. A 75-g OGTT will be performed at baseline and annually. Serum 25(OH)D levels will be measured by using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system<sup>34</sup> at baseline and annually. The system consists of a Waters Alliance 2795 HPLC interfaced to a Xevo TQ-S (Waters, Milford, MA). Bone mineral density (BMD) is not an obligatory measurement but will be evaluated upon request from participants. If fasting plasma glucose (FPG)  $\geq 126$  mg/dl or HbA<sub>1c</sub>  $\geq 6.5\%$  ( $\geq 48$  mmol/mol) is identified, a 75-g OGTT will be performed within 4 weeks. When HbA<sub>1c</sub>  $\geq 6.5\%$  ( $\geq 48$  mmol/mol) and at least one of the following is recognized, the participant will be diagnosed with diabetes: FPG  $\geq 126$  mg/dl, 75-g OGTT 2h-PG  $\geq 200$  mg/dl, or casual plasma glucose (PG)  $\geq 200$  mg/dl<sup>35</sup>. The participants with diabetes will be excluded from the followed-up data analysis. Conversely, when the participants achieve normoglycemia, defined as meeting all three glycemic criteria [FPG  $< 110$  mg/dl, 2h-PG  $< 140$  mg/dl, and HbA<sub>1c</sub>  $< 6.5\%$  ( $< 48$  mmol/mol)] or both FPG  $< 100$  mg/dl and HbA<sub>1c</sub>  $< 5.7\%$  ( $< 42$  mmol/mol)<sup>36</sup> are recorded successively at least twice, the date will be recorded, and they will be followed-up as analysis subjects until diabetes

onset. The follow-up period for all participants is 144 weeks.

### Primary endpoint

The primary endpoint is the hazard ratio of type 2 diabetes onset during 144 weeks of the study period: Active vitamin D group vs. Control group. We consider that the comparison of the treatment effect on type 2 diabetes onset between the two groups is the best measurement to evaluate the improvement of insulin resistance and glucose tolerance.

### Secondary endpoints

- 1) Improving ratio from IGT to normoglycemia at 48, 96, and 144 weeks.
- 2) Hazard ratios and 95% confidence intervals (CI) of type 2 diabetes onset in each subgroup at baseline: age ( $\geq$  /  $<$ 65 years), sex (men/women), presence or absence of hypertension (systolic  $\geq$  140 mmHg and/or diastolic  $\geq$ 90 mmHg), dyslipidemia (LDL-cholesterol  $\geq$ 140 g/dl and/or triglycerides  $\geq$ 150 mg/dl), obesity (body mass index  $\geq$  /  $<$ 25 kg/m<sup>2</sup>), family history of diabetes, FPG ( $\geq$  /  $<$ 110 mg/dl), 2h-PG ( $\geq$  /  $<$ 170 mg/dl), 25(OH)D<sub>3</sub> ( $\geq$  /  $<$ 25 ng/ml), homeostasis model assessment of insulin resistance (HOMA-R) ( $\sim$ 1.6 / 1.61–2.49 / 2.5~), and insulinogenic index ( $\geq$  /  $<$ 0.4).
- 3) Hazard ratios and 95% CI of type 2 diabetes development after adjusting for confounding factors: age ( $\geq$  /  $<$ 65 years), sex (men/women), presence or absence of hypertension (systolic  $\geq$ 140 mmHg and/or diastolic  $\geq$ 90 mmHg), dyslipidemia (LDL-cholesterol  $\geq$ 140 g/dl and/or triglycerides  $\geq$ 150 mg/dl), obesity (body mass index  $\geq$  /  $<$ 25 kg/m<sup>2</sup>), family history of diabetes, FPG ( $\geq$  /  $<$ 110 mg/dl), 2h-PG ( $\geq$  /  $<$ 170 mg/dl), 25(OH)D<sub>3</sub> ( $\geq$  /  $<$ 25 ng/ml), HOMA-R ( $\geq$  /  $<$ 2.5), insulinogenic index ( $\geq$  /  $<$ 0.4).
- 4) Hazard ratios of the incidence of adverse events
- 5) Amount and percentage change in HOMA-R during the 144 weeks
- 6) Changes in serum levels of the receptor activator of the nuclear factor kappa B ligand (RANKL), osteoprotegerin, osteocalcin, and leptin during 48 weeks and the association of these items with insulin resistance [HOMA-R and quantitative insulin sensitivity check index (QUICKI)]

**Statistical analyses**

Intention-to-treat population comprises all participants who undergo randomization and receive at least one dose of study drug, and will be analyzed for the assessment of effectiveness and safety. In this analysis plan, the data set is called the full analysis set. Per protocol set comprises all participants who undergo randomization and have no violation of inclusion and exclusion criteria, and this set will be analyzed as the secondary population in the sensitivity analysis. When summing data in each specified period, those data that are obtained beyond an acceptable range of the prescribed observation date or obtained by other than the default method or condition will be treated as missing data. Basic statistics (mean, standard deviation) are calculated for all continuous variables in each treatment arm and each visiting time. If necessary, basic statistics are calculated as a whole. As to FPG, HbA1c, and other laboratory data, differences between the values at baseline and those taken every 12 weeks, and their percent changes from baseline are calculated. Basic statistics, standard errors, or 95% CI are also calculated in these items. The distribution will be summarized in contingency tables for each treatment arm and each visiting time for all categorical variables. All significance tests and CI were two-sided and performed or constructed at the 5% significance level.

**Sample size**

Sample size was calculated with an 80% power ( $1-\beta$ ), a 0.05  $\alpha$  error, and a two-sided test for Log-rank test in Kaplan-Meier survival analysis. Based on our preliminary study (unpublished), we assumed that the cumulative incidence of type 2 diabetes in the control group is 23.14% (8.4% annually) and that in the active vitamin D group is 14.81% (5.2% annually) during 3 years. Thus, the relative risk reduction and drop-out ratio are assumed at 36% and 7%, respectively. As a result, 375 participants are required in each group (total 750).

**Interim analyses**

According to the incidence of diabetes, it is assumed that at least 133 participants will develop diabetes

1 during the study period. The total number of interim analyses will be two. When 1 year has passed after  
2  
3 the registration of the last participant, the first interim analysis will be conducted. Simultaneously, the  
4  
5 drop-out ratio of participants will be confirmed. If it is more than 3% per year, recruitment of trial  
6  
7 participants will be conducted again. The second interim analysis will be done when 80 participants  
8  
9 develop diabetes, that is, 60% of the expected diabetes incidence. Regarding the primary endpoint, when  
10  
11 the difference, determined by the O'Brien-Fleming method, between the both groups is  $< 0.0005$  (in first  
12  
13 interim analysis),  $< 0.014$  (in the second interim analysis), or  $< 0.045$  (this is not in an interim analysis but  
14  
15 the final analysis)<sup>37</sup>, early completion of the trial will be decided by the independent Data Monitoring  
16  
17 Committee.  
18  
19  
20  
21  
22  
23  
24

### 25 **Trial management and independent committees**

26 The Trial Steering Committee (Chair: GS) will meet on a termly basis to review status of the overall program,  
27  
28 including trial progress. In the case that they amend the protocol for a legitimate reason, they shall submit a  
29  
30 report about the amendment to the principal investigator and IRB as soon as possible for their review and  
31  
32 approval. The decision of the amendment must be informed to all authorized study personnel immediately.  
33  
34

35 The Data Collection Center will collect the accumulating outcome data from all trial participating  
36  
37 institutions. The staff member of the center will clean up and aggregate the data but won't know which group a  
38  
39 participant belongs to.  
40

41 The independent Data Monitoring Committee (Chair: TI) will audit the conduct of the trial with safety  
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43 and ethics review regularly and conduct the interim analyses. When the trial is finished or the independent  
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45 Data Monitoring Committee adjudicate the trial completion according to the result of the interim analyses,  
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47 the final dataset will be directly sent to a fully independent Data Analyses Committee (Chair: SM). The  
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49 data will be analyzed for all endpoints. Any investigators other than the Data Analyses Committee cannot  
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51 access the data.  
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### 57 **Ethical considerations and dissemination**

This study is being conducted according to Good Clinical Practice (GCP) and the Declaration of Helsinki<sup>38</sup>. It is carried out under the surveillance and guidance of the independent Data Monitoring Committee in compliance with the ICH-GCP guidelines. The study protocol has been approved by the Institutional Review Board at Kokura Medical Association, University of Occupational and Environmental Health, and Fujisawa City Hospital. A written informed consent is obtained from each participant before study participation. Participants are informed of their right to withdraw from the study at any time. This study was registered at the University Hospital Medical Information Network clinical trials registry (UMIN000010758). The study results will be presented at national and international conferences, and submitted for publication in peer-reviewed journals.

DISCUSSION

There is increasing interest in the potential health benefits by taking vitamin D supplements or active vitamin D formulations. Recent research suggests that vitamin D deficiency may cause not only bone diseases but also a range of non-skeletal diseases, such as cardiovascular diseases, cancers (eg. colorectal, breast), type 2 diabetes, infectious diseases (eg. influenza, common cold, tuberculosis), and autoimmune diseases (eg. multiple sclerosis, type 1 diabetes)<sup>9,39</sup>. However, most of these data come from observational studies, and clinical trial data on the effects of vitamin D are scarce and inconsistent<sup>31-33</sup>. Hence, we focused on the effectiveness of eldecalcitol, active vitamin D, on the incidence of type 2 diabetes among individuals at high risk for diabetes and designed the protocol of DPVD study. This study will provide valuable information, both on the incidence of type 2 diabetes and the improvement to the normal glucose state from IGT.

As a result of necessary compromises of study design, the DPVD study protocol has a number of strengths and weaknesses. Because this study consists of only Japanese participants, it is unknown whether the results of this study are adapted to other ethnicity. Several large-scale clinical trials to examine the benefits of vitamin D in a number of chronic diseases has recently been started around the

world: U.S., Finland, New Zealand, 8 European cities, and U.K.<sup>39,40</sup> The primary endpoints are cancer, cardiovascular disease, respiratory disease, infection, hypertension, bone fracture, cognitive function, and longevity. However, there is no trial of which primary endpoint is diabetes. As compared with these trials in which the number of participants is 2,000 to 20,000, that in our study is 750. However, our study is not considered to be inferior to others because of the following reasons. In other trials, primary endpoints are diverse; study participants are almost healthy person; there is no regular outpatient follow-up by a physician; vitamin D supplements are delivered by a mail once a year. Whereas, in our study, there is one primary endpoint; study participants are at high risk for diabetes; all participants visit clinics to be followed by physicians every 3 months; they are prescribed active vitamin D formulations at that visit. Therefore, the drug compliance and the follow-up ratio in our study will be better than those in other trials; additionally, the sample size of our study, 750 participants, will be enough to evaluate the primary endpoint.

Although vitamin D supplements are chosen to be used in most of other trials, active vitamin D formulation is chosen in our study. Humans get vitamin D from exposure to sunlight, from their diet, and from dietary supplements. Vitamin D from skin and diet is metabolized in the liver to 25-hydroxynitamin D; 25-hydroxynitamin D is metabolized in the kidneys by the enzyme 25-hydroxynitamin D-1 $\alpha$ -hydroxylase to its active form, 1,25-dihydroxynitamin D. Vitamin D itself does not have physiological effect but 1,25-dihydroxynitamin D has : it promotes intestinal calcium absorption in the small intestine and calcium resorption from bone by interacting with the vitamin D receptor to enhance the expression of the epithelial calcium channel and calcium- binding protein<sup>11</sup>. As a result, the administration of active vitamin D rather than vitamin D leads to expression of these physiological effects more effectively and promptly on not only bone and calcium metabolism but also non-skeletal tissue and glucose metabolism. Hence, we chose active vitamin D formulation in the present study. In conclusion, results from this study will provide a clearer picture of the risk and benefits of eldecaciol (active vitamin D) for prevention of type 2 diabetes, and contribute to the resolution of a debate “whether vitamin D and active vitamin D is panacea for glucose metabolism<sup>41</sup>.”

**Current study status**

The DPVD study began recruiting participants in June 2013 and we are still collecting data. It is expected to be reported in 2017.

**Acknowledgements**

We are grateful to all study participants and all staff of this study for recruiting the participants and contributing to the accurate recording of trial data.

**Contributors**

TK, GS, SM, and FK conceived and designed the study. TK drafted the protocol of the study and organized study implementation. TK, GS, TI, SM, FK, YO, and YT refined the study protocol and study implementation. SM and FK conducted the statistical analyses. TK, GS, TI, SM and YT have drafted the manuscript. All authors have read and approved the final version of the manuscript.

**Funding**

This study received an unrestricted grant from Kitakyushu Medical Association, and was supported by Chugai Pharmaceutical Co., Ltd. and Taisho-Toyama Pharmaceutical Co., Ltd. However, these companies are not involved in the design, conduct, analysis, or reporting of the study.

**Conflict of interest**

The authors declare that they have no conflict of interest.

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**Table 1. Eligibility criteria for participation in DPVD study**

Diagnostic criteria of IGT	FPG < 126 mg/dl, 75-g OGTT's 2h-PG is $140 \leq 2h-PG < 200$ mg/dl, and HbA1c < 6.5% (<48 mmol/mol). Additionally, he/she should not be medicated with any antidiabetic drug.
Inclusion criteria	Men and women aged $\geq 30$ years Individuals who are diagnosed with IGT. Serum calcium (corrected value): < 11.0 mg/dl
Exclusion criteria	Individuals who have participated in other clinical trials. Individuals who have been treated with active vitamin D, vitamin D supplement, and/or calcium preparation during last three months. Individuals who have already been diagnosed with type 1 or 2 diabetes. Individuals who have already been initiated with a drug treatment for pre-diabetes. Individuals who are pregnant or have severe diseases, such as renal insufficiency (serum creatinine > 1.5 mg/dl), hepatic insufficiency, psychosis, collagen disease, heart diseases, cerebrovascular diseases. A sub-investigator decides which participants' conditions are inappropriate and preclude his/her participation in the study.

IGT, impaired glucose tolerance; FPG, fasting plasma glucose; 75-g OGTT, 75-g oral glucose tolerance test; 2h-PG, 2-hour plasma glucose.

**Table 2. Outline of the study schedule**

	enrollment	allocation	12, 24, 36 weeks	48 weeks	60, 72, 84 weeks	96 weeks	108, 120, 132 weeks	144 weeks
Time point	-t <sub>1</sub>	t <sub>0</sub>	t <sub>1</sub> , t <sub>2</sub> , t <sub>3</sub>	t <sub>4</sub>	t <sub>5</sub> , t <sub>6</sub> , t <sub>7</sub>	t <sub>8</sub>	t <sub>9</sub> , t <sub>10</sub> , t <sub>12</sub>	t <sub>13</sub>
Enrollment								
Eligibility screen	X							
Informed consent	X							
Allocation		X						
Interventions								
Active vitamin D group								
Eldecalcitol 0.75µg /day								
Control group								
Placebo 1 capsule /day								
Assessments								
Drug compliance			X	X	X	X	X	X
Body height, weight, and blood pressure	X		X	X	X	X	X	X
Blood tests	X		X	X	X	X	X	X
75-g OGTT	X			X		X		X
25(OH)D	X			X		X		X
Bone Mineral Density *		X		X		X		X
Additional laboratory check †		X		X				
Safety monitoring								
Adverse event reporting								

\* Bone mineral density is measured by using dual-energy X-ray absorptiometry at baseline and every 48weeks if a participant wishes.

† Additional laboratory check includes serum levels of receptor activator of nuclear factor kappa B ligand (RANKL), osteoprotegerin, osteocalcin, and leptin.

75-g OGTT, 75-g oral glucose tolerance test; 25(OH)D, 25-hydroxy vitamin D.

Table 3. Blood tests

Sampling period	Analysis
Baseline and every 12-week	Fasting plasma glucose or Casual plasma glucose, HbA1c, IRI (glucose tolerance)
	White blood cell, Red blood cell, Hematocrit, Hemoglobin, Platelet (blood counting)
	Natrium, Potassium, Chlorine, Calcium, Phosphorus, Magnesium (serum electrolytes)
	LDL-cholesterol, HDL-cholesterol, Triglycerides (lipids)
	AST, ALT, $\gamma$ -GTP, Proteins, Albumin (liver function)
Baseline and every 48-week	Blood urea nitrogen, Creatinine, Uric acid (renal function)
	75-g OGTT includes IRI (glucose tolerance)
	25(OH)D (vitamin D level)
Baseline and 48-week	RANKL, Osteoprotegerin, Osteocalcin, Leptin (additional laboratory check)

HbA1c, glycated hemoglobin; IRI, immunoreactive insulin; LDL, low density lipoprotein; HDL, high density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase;  $\gamma$ -GTP,  $\gamma$ -Glutamyltranspeptidase; 75-g OGTT, 75-g oral glucose tolerance; 25(OH)D, 25-hydroxy vitamin D; RANKL, receptor activator of nuclear factor kappa B ligand.



## 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	___1___
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	___2 and 11___
	2b	All items from the World Health Organization Trial Registration Data Set	___2___
Protocol version	3	Date and version identifier	___N/A___
Funding	4	Sources and types of financial, material, and other support	___13___
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	___1 and 11___
	5b	Name and contact information for the trial sponsor	___4___
	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	___13___
	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)	___11___

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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	3
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	4
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)	4

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	4
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)	4 and Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	5, 7, and Table 2
	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)	6
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	2 and 6
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
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	8
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)	7 and Table 2

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	____9____
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	____N/A____

### Methods: Assignment of interventions (for controlled trials)

#### Allocation:

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	____5____
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	____5____
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	____5____
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	____5____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	____5____

### Methods: Data collection, management, and analysis

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	____7 and 10____
	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	____9____

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3	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	___1 and 10___
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7	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	___9___
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10		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	___9___
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12		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)	___9___
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16	<b>Methods: Monitoring</b>			
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18	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	___9 and 10___
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23		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	___9___
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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	___8___
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29	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	___10___
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33	<b>Ethics and dissemination</b>			
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35	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	___11___
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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)	___10___
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Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	_____5_____
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	_____N/A_____
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	_____10_____
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_____13_____
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	_____10_____
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	_____N/A_____
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	_____11_____
	31b	Authorship eligibility guidelines and any intended use of professional writers	_____13_____
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____N/A_____
<b>Appendices</b>			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	_____N/A_____
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	_____Table 3_____

\*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.

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

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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>8</u>
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	<u>10</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>14 and 29</u>
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	<u>N/A</u>
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	<u>13</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>15 and 21</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>18 and 20</u>

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>21 and 29</u>
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	<u>N/A</u>
<b>Methods: Assignment of interventions (for controlled trials)</b>			
Allocation:			
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>11</u>
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>10</u>
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	<u>11</u>
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	<u>11</u>
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	<u>11</u>
<b>Methods: Data collection, management, and analysis</b>			
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>13</u>
	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>25</u>

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	10
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	26
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	26
	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)	24
<b>Methods: Monitoring</b>			
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	15
	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	27
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	15
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	15
<b>Ethics and dissemination</b>			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	1 and 9
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)	29

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
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	10
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	28
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	N/A
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	15
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	30
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	N/A (We have a Japanese version.)
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	18

\*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" license.