“CASCADES” CLINICAL TRIAL PROTOCOL SUMMARY

"A randomized, open-label, clinical trial evaluating the anti-atherosclerotic efficacy of selected antidiabetic drugs in patients with coronary artery disease and pre-diabetic conditions. CASCADES Trial"

Date and version number	Version 2.1 dated 06/09/2024
Protocol symbol	CSDS.IV/VIII/2023
EU CT number	2023-508525-27-00
Type of test	non-commercial clinical trial
Center	Cardinal Stefan Wyszyński National Institute of Cardiology – National Research Institute
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Funding organization	Medical Research Agency, ul. Stanisława Moniuszki 1A, 00-014 Warsaw
Project management	Clinical Research Support Center, Cardinal Stefan Wyszyński National Institute of Cardiology – National Research Institute, 42 Alpejska St., 04-628 Warsaw, tel. +48 22 343-40-80; e-mail: kkotlinska@ikard.pl

Study title	A randomized, open-label, clinical trial evaluating the anti-atherosclerotic efficacy of selected antidiabetic drugs in patients with coronary artery disease and pre-diabetic conditions. CASCADES Trial.
Acronym	CASCADES
EU CT:	2023-508525-27
Sponsor	Cardinal Stefan Wyszyński National Institute of Cardiology – National Research Institute, ul. Alpejska 42, 04-628 Warsaw.
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Research center	Cardinal Stefan Wyszyński National Institute of Cardiology – National Research Institute, ul. Alpejska 42, 04-628 Warsaw. Single-center study.
Study rationale	The purpose of the study is to compare the anti-atherosclerotic efficacy of oral treatment with a GLP-1 analogue (semaglutide) or an SGLT-2 (so-called “flozin”) inhibitor (dapagliflozin) versus routine treatment (metformin) in patients with pre-diabetes and diagnosed coronary artery disease at 24 months. The diagnosis of coronary artery disease will be defined as the presence of coronary atherosclerosis confirmed by coronary artery computed tomography (coronary CT).
Clinical phase	IV
Study methodology	Randomized controlled, open, with two arms of therapeutic intervention
Measurements and procedures	Evaluation of coronary arteries by computed tomography scanning of coronary arteries with contrast Pharmacological intervention: flozin 10 mg vs. semaglutide 3 mg – 7 mg, 14 mg vs. metformin 500 mg – 1000 mg (1:1:1) Physical examination Laboratory tests (venous blood draw, general urinalysis) Measurement of weight, height, blood pressure Assessment of body composition by bioimpedance Resting 12-lead electrocardiogram Non-pharmacological control of coronary heart disease risk factors (dietary, anti-smoking and appropriate physical activity counseling)

Inclusion and exclusion criteria	Inclusion criteria: • Age 18 – 80 • Diagnosed coronary artery disease (coronary artery stenosis of at least 20% with a reference diameter of >2.5 mm or status after percutaneous coronary revascularization procedure found on coronary CT scan) • Coronary CT scan performed <3 months after inclusion in the study, at least of good quality • Pre-diabetic status defined as fasting blood glucose 100-125 mg% or Hba1c 5.70-6.49% (measurement documented at the screening/randomization appointment or within 30 days prior to the screening/randomization appointment) or documented, positive result of an oral glucose load test (fasting blood glucose 100-125 mg% and 140-199 mg% 2h after a 75 g oral glucose load) performed up to 30 days before the screening/randomization appointment • Stable treatment and control of cardiovascular risk factors, including dietary and lifestyle management for at least 4 weeks • Willing and able to give informed consent to participate in the study • Willing and able, according to the researcher, to comply with all the requirements of the study Exclusion criteria: • Severe valvular defect • Clinical condition requiring surgical treatment of coronary artery disease • Status after coronary artery bypass surgery • Diagnosed diabetes or Hba1c>=6.5% at screening/randomization appointment • Other severe medical conditions requiring scheduled hospital treatment at the time of the study • Severe musculoskeletal conditions requiring specific rehabilitation recommendations • Diagnosed heart failure • Presence of an artificial valve, cardiac pacing system or other implantable device (such as a cardioverter defibrillator)

	• Severe arrhythmia/unexplained loss of consciousness • Other contraindications to physical activity • No consent to participate in the study • Use of glucose-lowering drugs other than metformin • Use of weight-loss drugs • Condition after bariatric surgery • Diagnosed liver disease or ALT, AST above three times the upper limit of normal at screening appointment • Uncompensated hyperthyroidism • Pancreatic cancer • Medullary thyroid cancer • History of anaphylactic shock after iodine contrast administration • Chronic kidney disease (eGFR <45 ml/min/1.73 m2) • History of pancreatitis or active pancreatitis • Body mass index (BMI) >40 kg/m2 • Pregnancy/lactation • Participation in another clinical trial • Other known contraindications to treatment with metformin, dapagliflozin or semaglutide
Sample size	300 patients
Statistical issues	The study is exploratory, and the primary analysis is designed to separately compare each of the two active groups (treatment: semaglutide or dapagliflozin) with the control group (routine treatment – metformin). According to the scientific literature, the size of the study group was calculated based on a separate comparison of each active group with the control group, without adjusting the power ratios (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7534018/). We hypothesized that the % change in the volume of non-calcified atherosclerotic plaque (final values versus initial values) in each active group differed from the control group. To determine the sample size, the following assumptions were made about treatment efficacy as measured by % change in non-calcified plaque volume compared to baseline values: Standard deviation of 2.5% for the primary variable. Treatment difference of at least 1.2% in non-calcified plaque in favor of each study drug compared with the control group.

	Taking into account the 7% of patients excluded from the study (no follow-up CT scan at month 24), a total sample size of 300 participants (282 after withdrawals), with 93 in the semaglutide group, 93 in the dapagliflozin group, and 93 in the control group, provides a power of 0.90 with a two-sided alpha level of 0.05.		
Planned study duration	Single participant observation period: 24 months Project start date 01/09/2023 Enrolment start date 01/06/2024 Completion of treatment and observation 01/06/2028 Project completion date 01/08/2029		
Planned enrolment period	01/06/2024 – 01/06/2026		
	Purposes	Endpoints	Time points
Co-primary	Evaluation of the effect of GLP-1 analogue treatment on coronary artery disease progression Evaluation of the effect of flozin treatment on the progression of coronary artery disease	Change in % volume of non-calcified atherosclerotic plaque in the coronary arteries assessed by coronary CT versus routine management (intention-to-treat) Change in % volume of non-calcified atherosclerotic plaque in the coronary arteries assessed by coronary CT versus routine management (intention-to-treat)	24 months 24 months

Secondary	Evaluation of the effect of each of the tested drugs vs. control group on progression of coronary artery disease	% change in volume of non-calcified atherosclerotic plaque assessed by coronary CT (as treated) between baseline and end of study	24 months
	Comparison of the effect of semaglutide vs. flozin on coronary artery disease progression	% change in volume of non-calcified atherosclerotic plaque assessed by coronary CT (intention to treat/as treated) between baseline and end of study	24 months
	Evaluation of the effect of each study drug vs. control group/comparison of the effect of semaglutide vs. flozin on progression of coronary artery disease	% change in volume of the entire atherosclerotic plaque assessed by coronary CT) (intention to treat/as treated) between baseline and end of study	24 months
	Evaluation of the effect of each of the tested drugs vs. control group/comparison of the effect of semaglutide vs. flozin on progression of coronary artery disease	% change in volume of individual components of atherosclerotic plaque assessed by coronary CT (intention to treat/as treated) between baseline and end of study	24 months
	Evaluation of the effect of each of the tested drugs vs. control group/comparison of the effect of semaglutide vs. flozin on progression of coronary artery disease	Conversion of non-calcified plaque to calcified plaque (change in % volume of calcified plaque relative to % volume of non-calcified plaque) assessed by coronary CT (intention to treat/as treated) between baseline and end of study	24 months

	Evaluation of the effect of each of the tested drugs vs. control group/comparison of the effect of semaglutide vs. flozin on CV risk expressed as the dynamics of high-risk features	1. Change in the number of high-risk atherosclerotic lesions defined as the presence of at least 2 high-risk features among: - Spotty calcifications - Low attenuation plaques (low attenuation plaque), i.e. plaque density < 30 HU - positive remodeling - napkin ring sign assessed by coronary TK (intention-to-treat/as treated) between baseline and end of study;	24 months
		2. Change in the Pericoronary Fat Attenuation Index assessed by coronary TK (intention-to-treat/as treated) between baseline and end of study	
	Evaluation of weight change, including body composition, in patients treated with semaglutide vs. patients treated with flozin	- weight - body mass index (BMI). - fat mass - skeletal muscle mass - Fat-To-Muscle Ratio (FMR). - Body Cell Mass (BCM) - Visceral Fat Area (VFA)	24 months
	Evaluation of waist-to-hip index (WHI) change in patients treated with semaglutide vs. patients treated with phlorizin	Change in waist-to-hip index (WHI) between baseline and end of study	24 months
	Evaluation of change in inflammatory parameters in patients treated with semaglutide vs. patients treated with phlorizin	Concentration of hsCRP	24 months

	Evaluation of change in lipid levels in patients treated with semaglutide vs. patients treated with flozin	- total cholesterol concentration - low-density lipoproteins (LDL) concentration - high-density lipoproteins (HDL) concentration - non-HDL cholesterol concentration - triglycerides concentration - lipoprotein A concentration	24 months
	Evaluation of change in the percentage of glycated hemoglobin (HBA1c) in patients treated with semaglutide vs. patients treated with flozin	Change in the percentage of glycated hemoglobin (HBA1c) between baseline and end of study	24 months
	Evaluation of change in the percentage of patients with normal blood pressure in patients treated with semaglutide vs. patients treated with flozin	Change in percentage of patients with normal blood pressure defined as systolic pressure <140 mmHg and diastolic pressure <90 mmHg between baseline and end of study	24 months
	Evaluation of change in the percentage of patients currently smoking tobacco or electronic cigarettes in patients treated with semaglutide vs. patients treated with flozin	Change in the percentage of patients currently smoking tobacco (cigarettes, pipe, cigar, tobacco heating products) or electronic cigarettes as defined in section 8.7.a between baseline and end of study	24 months
	Evaluation of compliance with physical activity recommendations in patients treated with semaglutide vs. patients treated with flozin	- Percentage of patients classified in the "high" physical activity category; - Percentage of patients classified in the "sufficient" physical activity category;	24 months

		- Percentage of patients classified in the "insufficient" physical activity category	
	Evaluation of dietary compliance in patients treated with semaglutide vs. patients treated with flozin	Change in the DASH Index between baseline and end of study	24 months
	Type 2 diabetes diagnosis	Number of patients diagnosed with diabetes- based on the criteria of the Polish Diabetes Association	24 months
	Evaluation of the onset of heart failure requiring hospitalization	Number of hospitalized patients for heart failure	24 months
	Number of unplanned hospitalizations	Number of unplanned hospitalizations	24 months
	Number of major cardiovascular events and strokes (MACCE: death/myocardial infarction/revascularization /stroke) separately and combined	Number of cardiovascular events and strokes (MACCE: death/myocardial infarction/revascularization /stroke)	24 months
	Homeostatic Model Change Assessment – Insulin Resistance (HOMA-IR).	Homeostatic Model Assessment – Insulin Resistance (HOMA-IR) Change.	12-24 months
	Evaluation of change in the concentration of selected oxidative stress markers	Change in concentration of selected oxidative stress markers: catalase, superoxide dismutase (SOD), Oxygen Radical Absorbance Capacity (ORAC), total antioxidant capacity (TAC)	12-24 months

Results	The study will evaluate the effect of treatment with flozin vs. semaglutide compared to treatment with metformin on the progression/regression of coronary atherosclerosis, change in plaque character, and control of cardiovascular risk factors in patients with non-obstructive coronary artery disease and pre-diabetic status
Interventions:	
Tested medicinal product #1	Dapagliflozin (Forxiga), 10 mg film-coated tablets, oral
Tested drug product #2	Semaglutide (Rybelsus), 3 mg tablets, 7 mg tablets, 14 mg tablets (target dose), oral
Other interventions	Dietary, anti-smoking and appropriate physical activity counseling
Comparator	Metformin (Formetic), 500 mg film-coated tablets or 1000 mg film-coated tablets, oral
Study schedule	Month and year of inclusion of the first participant: June 2024 Month and year of completion of the last participant's participation: June 2028 A pictorial diagram of the study is shown in Figure 1. A detailed schedule of appointments is provided in Section 8.6 (Table 1).

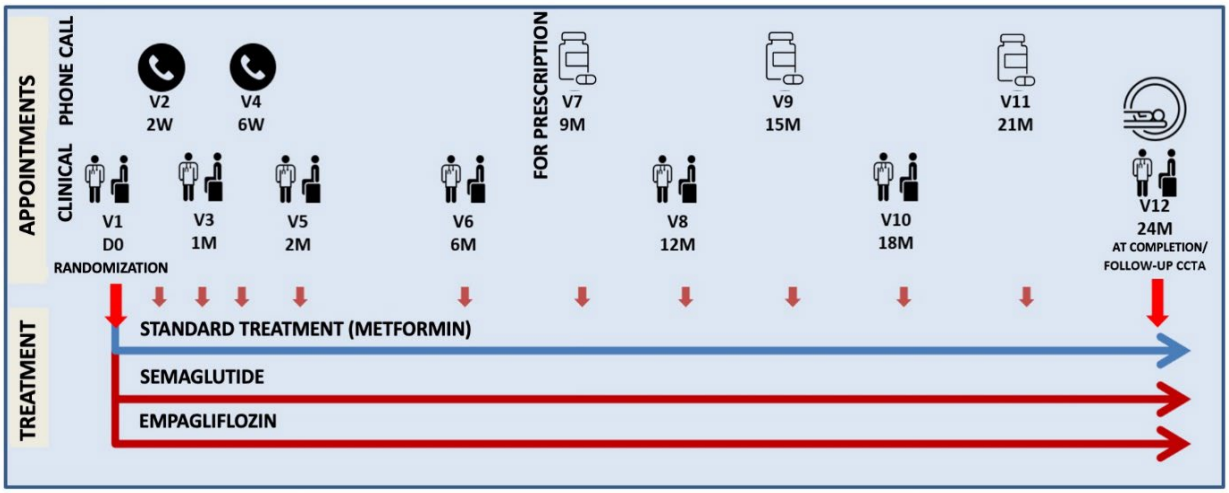

Figure 1. Study diagram overview by appointments

Detailed schedule of appointments

Appointment	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**
Time window (D-Day/W-Week/M-Month)	D0	W2 (±3 D)	M1 (±3 D)	W6 (±3 D)	M2 (±3 D)	M6 (±3 D)	M9 (±3 D)	M12 (±9D)	M15 (±9D)	M18 (±9D)	M21 (±9D)	M24 (±9D)
Clinical (K) / Prescription (R) / Telephone (T).	**K**	**T**	**K**	**T**	**K**	**K**	**R**	**K**	**R**	**K**	**R**	**K**
Cardiology consultation	●		●		●	●		●		●		●
Physical examination	●		●		●	●		●		●		●
Presenting the patient with an offer to participate in the study. Discussing the risks and procedures involved in participating in the study. Providing the patient with an informed consent form for participation in the study	●											
Obtaining consent to participate in the study	●											

Performing an ECG	●					●		●		●		●
Collection of blood for determination of blood count, creatinine/GFR, lipid profile, glucose, Hba1c, insulin, lipoprotein A, ALT, AST, determination of TSH in selected patients (appointment V12); determination of hsCRP, HOMA-IR oxidative stress markers (appointment V1, V8, V12)	●		●		●	●		●		●		●
General urinalysis	●		●		●	●		●		●		●
Taking measurements of weight, height, blood pressure, body composition, abdominal/hip circumference measurements	●		●		●	●		●		●		●
Assessment of comorbidities, diet (history)	●		●		●	●		●		●		●
Assessment of physical activity levels (e.g., using an app that measures physical activity levels on the patient's smartphone or other mobile device)	●		●		●	●		●		●		●
Advice on dietary	●		●		●	●		●		●		

goals and how to achieve them												
Advice on optimizing physical activity levels	●		●		●	●		●		●		
Advice on how to quit smoking	●											
Quality-of-life assessment – EQ5D-5L,Seattle Angina Questionnaire~~,~~	●		●		●	●		●		●		●
Randomization – allocation to the arm (experimental or control, 2:1) and, in the case of the experimental arm, to the appropriate drug group (flozin or semaglutide, 1:1), dispensing of the drug by support staff	●											
Transfer of CT imaging data records to the central imaging laboratory (Core Lab) for independent analysis	●											●
Evaluation of the occurrence of endpoints (according to the description of endpoints, Chapter 6)		●	●	●	●	●	●	●	●	●	●	●
Collection of packages of drugs assigned during the previous appointment. Dispensing of new			●		●	●		●		●		●

drugs (except V12)												
Assessment of the regularity of the patient's drug intake		●	●	●	●	●	●	●	●	●	●	●
Evaluation of the tolerability of the assigned drugs, taking into account potential side effects		●	●	●	●	●	●	●	●	●	●	●
Reminder to follow dietary recommendations and maintain adequate levels of physical activity		●		●			●		●		●	
Pregnancy test	●											●
Coronary CT study												●
Providing recommendations for treatment after participation in the study												●

Table 1. Detailed scope of activities and procedures performed during appointments