CES1 Crossover Trial of Clopidogrel and Ticagrelor

NCT03161678

March 5, 2024

STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

The purpose of this investigation was to conduct a randomized crossover study of clopidogrel and ticagrelor in participants recruited by carboxylesterase 1 (*CES1*) genotype (G143E and rs7498748). Extensive phenotyping including *ex vivo* platelet aggregometry performed pre- and post-drug administration was performed in order to assess the interaction of genotype and drug choice on on-treatment platelet function. Specifically, we enrolled 89 healthy Amish participants based on *CES1* genotype (17 G143E carriers, 38 rs7498748 minor allele carriers, and 34 controls who did contain the minor alleles of either of these variants). All of these individuals underwent a randomized crossover study of clopidogrel (75 mg per day for 7 d) and ticagrelor (90 mg twice daily for 7 d), with at least a 14-day washout period between drug interventions. Platelet aggregation studies and other clinical information were recorded before and after each intervention.

Home visit to screen for eligibility: A research nurse and an Amish community liaison visited potential research subjects in their homes. The nurse explained the study and obtained informed consent. If a research subject consented, the nurse obtained a medical history and screening laboratory tests to assess eligibility. Briefly, participants were excluded if they were pregnant, currently breastfeeding, had history of a bleeding disorder or major spontaneous bleed, hypertensive, creatinine levels > 2.0 mg/dL, ALT or AST > 2 x the upper limit of normal, hematocrit < 28%, TSH < 0.4 or >5.5 mIU/L, platelet count > 500,000 or < 75,000, currently taking clopidogrel, ticagrelor, prasugrel, or other anti-coagulant, history of cardiovascular disease or diabetes, was taking vitamins or other supplements and was unwilling or could not safely, in the opinion of the study physician, discontinue their use at least 1 week prior to protocol initiation.

Subjects were informed that to participate in this study, they had to discontinue for a period starting 7 days prior to the initial clinic visit any vitamins, supplements and medications that might affect the results of the study. Specifically, if a subject is on medications that may potentially interfere with the planned intervention, he/she was instructed to consult with his/her physician regarding discontinuing such therapy prior to study enrollment. Alternatively, with the subject's permission, the study physician contacted the subject's physician to discuss and obtain permission for medication withdrawal. If in the opinion of the subject's physician and the study physician, discontinuation of medications for the duration of the study did not pose any undue health risks, medications were discontinued one week before the first clinic visit. If a medication required tapering, this commenced two weeks prior to the first clinic visit. Similarly, all vitamins (except daily multivitamins) and other supplements were withdrawn for 7 days prior to the first clinic visit. If the research subject was on anti-platelet medications, these agents were withdrawn 14 days prior to the first clinic visit.

Female research participants were informed that they could not participate in this study if they are pregnant. All female subjects had an assessment of child-bearing potential. Pregnancy status was determined by self-report at the screening visit. A urine pregnancy test was performed at the clinic visits #1 and #3 prior to drug administration.

Following review of screening information and lab results, eligibility was confirmed. Eligible participants completed studies during the course of four visits to the Amish Research Clinic in Lancaster, PA.

Randomization: Prior to Clinic Visit #1, enrolled subjects were randomized (1:1) to either the clopidogrel or ticagrelor intervention. Clinical staff, blinded to *CES1* genotype, were notified of the order of drug intervention though the delivery of a sealed, opaque envelope from a research team member who was also blinded to participant genotype. Assignment envelopes were shuffled prior to study initiation and delivered sequentially. Participants who started with the clopidogrel intervention underwent the ticagrelor intervention after the prerequisite washout period and vice versa.

Clinic Visit #1 (Baseline Platelet Function): Research subjects were transported to the Amish Research Clinic after an overnight fast where heights, weights, and vital signs were measured. Women of childbearing age completed a pregnancy test. A catheter was placed, and fasting blood samples were obtained to assess circulating lipid levels (LDL, HDL, and triglycerides), evaluate baseline measures of agonist-stimulated platelet aggregation, and for sample banking. Participants were then observed while taking their first dose of clopidogrel or ticagrelor (based on randomization procedure), discharged to home with additional tablets, and

instructed to take the appropriate dose (75 mg/ daily for clopidogrel, 180 mg/d for ticagrelor) at the same time each day. A participant logbook was given to each participant to record their daily doses

Clinic Visit #2 (On-Clopidogrel Platelet Function): On the 7th day, subjects returned to the clinic, observed taking their last dose, and fasting blood samples were drawn 1-hour post-dose for measurement of agonist-stimulated platelet aggregation and sample banking. Drug adherence was assessed by pill count and participant logbook. Each participant then underwent a 14-day washout period to restore basal platelet function, consistent with the average lifespan of a platelet (7 – 10 days).

Clinic Visit #3 (Repeat Measures of Baseline Platelet Function): After the 14-day washout period, research subjects again were transported to the Amish Research Clinic in the fasting state where they were weighed and their vital signs measured. Reproductive age women underwent a 2nd pregnancy test. Blood samples were obtained for repeat measures of baseline platelet function and sample banking. Participants were then observed while taking their first dose of clopidogrel or ticagrelor (whichever one was not administered during the first intervention period), discharged to home with additional tablets, and instructed to take the appropriate dose at the same time each day. A participant logbook was given to each participant to record their daily doses.

Clinic Visit #4 (On-Ticagrelor Platelet Function): On the 7th day, subjects returned to the clinic, observed taking their last dose, and fasting blood samples were drawn 1-hour post-dose for measurement of agonist-stimulated platelet aggregation and sample banking. Drug adherence was assessed by pill count and participant logbook.

Assessment of Platelet Function: at each clinic visit, platelet-rich plasma (PRP) was isolated from fasting blood samples drawn into 3.2% citrate-anticoagulated tubes (Becton-Dickinson, Franklin Lakes, NJ) and platelet counts were adjusted to 200,000 platelets/μl using platelet-poor plasma. Platelet function was assessed by optical aggregometry using a PAP8E Aggregometer (Bio/Data Corporation, Horsham, PA) according to the manufacturer's instructions after stimulation with 20 μM ADP and expressed as the maximal percentage change in light transmittance using platelet-poor plasma as a referent.

Distributions of summary statistics (e.g. age, sex, change in platelet function) were generated using SAS (Cary, NC). Change in clopidogrel-induced platelet aggregation in response to ADP was calculated by subtracting observed post-clopidogrel maximal platelet aggregation values from baseline (i.e. pre-drug) values. Similarly, change in ticagrelor-induced platelet aggregation in response to ADP was calculated by subtracting observed ticagrelor maximal platelet aggregation values from baseline (i.e. pre-drug) values.

The primary endpoint of this investigation was to determine within each drug group if *CES1* genotype is associated with change in platelet aggregation. Change in platelet aggregation, defined as the relative differences in platelet aggregation between baseline and day 7 of a given drug intervention, was calculated as: Change = [(MPA $_{baseline}$ – MPA $_{day\ 7}$), where MPA = maximal platelet aggregation. Consideration of relevant biological variables that influence platelet aggregation and/or drug response were explored in our statistical models. Specifically, the effect of *CES1* genotype on agonist-stimulated change in platelet aggregation was calculated using a variance component method under an additive or dominant model that simultaneously adjusted for age, sex, body mass index (BMI), and relatedness among study participants. Relatedness among participants were accounted for by including a polygenic component as a random effect. Briefly, a model for the polygenic component was established by constructing a relationship matrix derived from the complete Amish pedigree structure available through published genealogical records maintained by the church.